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Exposure to Polycyclic Aromatic Hydrocarbons and Early Cardiovascular Effects

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Exposure to Polycyclic Aromatic Hydrocarbons and Early Cardiovascular Effects

A dissertation submitted in partial satisfaction of

the requirements for the degree

Doctor of Environmental Health Sciences

by

Yan Lin

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ABSTRACT OF THE DISSERTATION

Exposure to Polycyclic Aromatic Hydrocarbons and Early Cardiovascular Effects

by

Yan Lin

Doctor of Environmental Health Sciences

University of California, Los Angeles, 2019

Professor Yifang Zhu, Co-Chair

Professor Jesus A Araujo, Co-Chair

Polycyclic aromatic hydrocarbons (PAHs) are a group of combustion-originated chemicals with two or more fused aromatic rings, which ubiquitously present in the ambient air. Substantial evidence indicates that PAHs cause cancers due to their carcinogenesis and mutagenesis, however, less understood is to what extent PAHs influences cardiovascular (CV) health. While previous animal studies have demonstrated that PAHs caused CV diseases, comparable evidence in humans is lacking. Most human studies on the relationship between PAHs and CV diseases were with a cross-sectional study design and yielded inconsistent results, which might be at least partially due to the lack of exposure contrast. Previous studies have documented a more than 10-fold difference in airborne PAHs between Los Angeles (< 10 ng/m^3) and Beijing (>100 ng/m^3), providing a dramatic exposure contrast among the international travelers between the two cities. This dissertation took advantages of a natural experiment among international travelers between Los

Angeles and Beijing to determine the early CV effects associated with PAHs exposures. This work is subdivided into the following five chapters: an introduction (Chapter 1), three chapters of original research (Chapters 2 - 4), and a discussion of the conclusions of the work (Chapter 5).

Chapter 2 evaluated the alteration of PAHs exposure among ten healthy human subjects who traveled between Los Angeles and Beijing in 2012 and explored the associated health effects. This pilot study has demonstrated elevated exposure to PAHs in Beijing as compared with Los Angeles and identified lipid peroxidation as one of the early CV effects that were associated with PAHs exposure. This chapter provide experimental basis for later works and justify larger studies in chapters 3 and 4.

Chapter 3 systematically tested a panel of circulating biomarkers indicative of systemic inflammation and oxidation in response to changes in PAHs exposures among 26 healthy international travelers in 2014 and 2015. The results indicated that traveling to Beijing led to increased lipid peroxidation and inflammation in associations with urinary PAHs metabolites. This study also suggested hydroxyeicosatetraenoic (HETE), hydroxyoctadecadienoic (HODE) acids, and paraoxonase 1 (PON1) enzymatic activities have potentials to serve as sensitive biomarkers to detect early CV effects induced by environmental exposures.

Chapter 4 extended previous works to 2017 and investigated the temporal trends of PAHs exposure in Los Angeles and Beijing. The results indicated that exposure to PAHs didn't change in Los Angeles, but significantly decreased in Beijing from 2012 to 2017. This study discussed the potential driving forces of the temporal trends in each city, aiming to achieve a better understanding on how chemical regulations influence human exposure to PAHs.

The dissertation of Yan Lin is approved.

Michael Jerrett

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2019

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LIST OF ABBREVAITION

8-OHdG	8-hydroxydeoxyguanosine
AhR	Aryl hydrocarbon Receptor
$ApoE^{-/-}$	Apolipoprotein E null
APPCAP	Air Pollution Prevention and Control Action Plan
BaP	Benzo(α)pyrene
BMI	Body Mass Index
BPA	Bisphenol A
CDC	The Centers for Disease Control and Prevention of U.S
CIMT	Carotid intima-media thickness
CO	Carbon Monoxide
CRP	C-Reactive Protein
CYP	Cytochromes P450
EH	Epoxide Hydrolase
FDR	False Discovery Rate
GBD	Global Disease Burden
GC-MS	Gas Chromatography and Mass Spectrometer
GST	Glutathione S-Transferase

HDL	High Density Lipoprotein
HETEs	Hydroxyeicosatetraenoic acids
HODEs	Hydroxyoctadecadienoic acids
HPLC	High-Performance Liquid Chromatography
ICC	Intra-individual Correlation Coefficient
LDL-$R^{-/-}$	Low Density Lipoprotein Receptor null
LOQ	Limit of Quantification
LOX	Lipoxygenase
IQR	Interquartile Range
MDA	Malondialdehyde
NAAQS	National Ambient Air Quality Standards
NAP	Naphthalene
NHANES	The National Health and Nutrition Examination Survey
NN-PAHs	Non-Naphthalene Polycyclic Aromatic Hydrocarbons
NO_2	Nitrogen Dioxide
O_3	Ozone
OH-BPs	Hydroxyl-biphenyl
OH-DBF	Hydroxy-dibenzofuran

OH-FLUs	Hydroxyl-fluorene
OH-NAPs	Hydroxy-naphthalene
OH-PAHs	Hydroxy-Polycyclic Aromatic Hydrocarbons
OH-PHEs	Hydroxyl-phenanthrene
OH-PYR	Hydroxy-pyrene
PAHs	Polycyclic Aromatic Hydrocarbons
PKU	Peking University, located in Beijing, China
PM	Particle Matter
$PM_{2.5}$	PM with aerodynamic diameter ≤ 2.5 μm
PON1	Paraoxonase 1
PUFAs	Polyunsaturated Fatty Acids
ROS	Reactive Oxygenated Species
SO_2	Sulfur Dioxide
SULT	Sulfotransferase
TBA	2-Thiobarbituric Acid
TSCA	Toxic Substances Control Act
UCLA	University of California, Los Angeles, located in Los Angeles, CA, U.S.
UGT	UDP-glucuronosyltransferase

UV	Ultraviolet
vWF	von Willebrand factor

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VITA

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1. **Lin, Yan**; Qiu, Xinghua*; Nu, Yu; Yang, Qiaoyun; Araujo, Jesus; Zhu,Yifang*. Urinary metabolites of polycyclic aromatic hydrocarbons and the association with lipid peroxidation: a biomarker-based study between Los Angeles and Beijing. ***Environ. Sci. Technol.*** 2016, doi: 10.1021/acs.est.5b04629
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3. **Lin, Yan**; Qiu, Xinghua; Liu, Jinming; Zhu, Yifang. Temporal Trends of Exposures to Polycyclic Aromatic Hydrocarbons and Bisphenol A during 2012-2017: A Comparison between China and the United States. The Joint Annual Meeting of the International Society of Exposure Science and the International Society for Environmental Epidemiology (ISES-ISEE 2018), August 2018, Ottawa, Canada (oral)

4. **Lin, Yan**; Qiu, Xinghua; Araujo, Jesus; Zhu, Yifang. A novel approach to apportion the primary and secondary sources of nitrated polycyclic aromatic hydrocarbons. The 35th Annual Conference of American Association for Aerosol Research (AAAR), October 2016, Portland, OR, USA (oral)

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1. BACKGROUD AND MOTIVATION

1.1. Air pollution promotes cardiovascular diseases worldwide.

The global burden of disease (GBD) project found that particulate matter (PM) was among the leading risk factors for mortality and lost years of healthy life worldwide, contributing to 4.2 million premature deaths in 2015.[1] A recent study re-analyzed the GBD data and found that the PM-induced global death might be up to 8.9 million.[2] In populous developing countries such as China, exposure to high levels of fine particles ($PM_{2.5}$, aerodynamic diameter $\leqslant$ 2.5 μm) is a serious public health problem which adversely affects not only 1.4 billion Chinese residents but also 60 million international visitors every year.[3] Most of $PM_{2.5}$ related deaths are due to ischemic cardiovascular (CV) and cerebrovascular diseases.[4] The Women Health Initiative Study demonstrated a 24% increase in the incidence of CV events and a 76% increase in CV mortality for every 10 $\mu g/m^3$ increase in the annual aver-age $PM_{2.5}$ level.[5] Increased CV risk includes the promotion of atherosclerosis and ischemic heart disease. This notion is supported by several epidemiological studies that demonstrate the association of PM exposure with carotid atherosclerosis in humans.[6–8] Animal studies suggest that these associations are likely to be causal.[9,10] Previous study in our laboratory has shown that apolipoprotein E null (Apo $E^{-/-}$) mice exposed to ultrafine particles (UFPs, diameter $\leqslant$ 0.1 μm) and $PM_{2.5}$ developed 55% and 24% greater aortic plaque area than mice exposed to filtered air, respectively,[10] however, the underlying mechanism is less understood.

1.2. $PM_{2.5}$ chemical composition and CV diseases.

$PM_{2.5}$ exposures in health effects studies are customarily assigned as undifferentiated particle mass concentrations. Albeit a large body of literature linking $PM_{2.5}$ exposures to CV health, little is known as to whether some components of the $PM_{2.5}$ mixture are of greater public concerns than

others. Recent epidemiologic studies suggested that combustion-originated $PM_{2.5}$ components such as polycyclic aromatic hydrocarbons (PAHs) are likely to play important roles in determining overall $PM_{2.5}$'s health effects.[4,11] PAHs are a group of air pollutants that contain two or more fused aromatic rings and mainly generated from incomplete combustion processes such as traffic emission and coal burning.[12] PAHs pose significant risks to human health as many of the individual PAHs are carcinogenic and mutagenic.[12] While the cardiovascular effects of PAHs were still not fully understood yet, previous studies have shown that benzo[α]pyrene (BaP), a five-ring PAH, promoted the progression of atherosclerosis in $ApoE^{-/-}$ mice.[13] In human studies, self-reported CV diseases and carotid intima-media thickness (CIMT) were positively associated urinary PAHs metabolites,[14,15] however, most of those studies are with a cross-sectional design, and it remains unclear whether those associations reflected a causal relationship.

1.3. Systemic oxidation and inflammation in PM/PAHs-induced CV diseases

Substantial evidence suggests that systemic oxidation and inflammation are central to PM/PAHs' ability to promote atherosclerosis.[16] Previous studies have found that exposure of $ApoE^{-/-}$ mice to diesel exhaust particles, rich in PAHs, leads to pro-oxidative effects in the lungs and in systemic tissues that are associated with the loss of high-density lipoproteins (HDL)'s antioxidant and anti-inflammatory ability.[17] Cross-sectional studies also found significant associations between long-term exposure to PM/PAHs and various biomarkers of systemic oxidation (e.g. urinary malondialdehyde (MDA) and circulating 8-isoprostane) and inflammation (e.g. C-reactive protein (CRP)).[18–21] Toxicological studies have suggested mechanisms by which PM/PAHs promote systemic oxidation, including the induction of mitochondrial dysfunction and deactivation of anti-oxidative enzymes.[11]

1.4. Lipoxygenase and paraoxonase 1 in PM/PAHs-induced system oxidation

In contrast to the frequently observed associations between long-term exposure to PM/PAHs and systemic oxidation, results from short-term exposures have been relatively inconsistent.[22] The lack of significant short-term effects might be due to the exposure duration or dose, timing of sample collections, suboptimal sensitivity of the biomarkers employed, or some combination of these factors. Therefore, it is important to identify new biomarkers that are sensitive and informative on pathogenetic mechanisms for the detection of early CV effects.

Lipoxygenases (LOX) and paraoxonase 1 (PON1) are pro- and anti-oxidative enzymes, respectively. Importantly, activation of LOXs and inhibition of PON1 also present pro-inflammatory effects and have been shown to exacerbate atherosclerosis in rodent models and human histological studies, [23–26] making their assessment attractive in the evaluation of PM/PAHs-induced early CV effects. Previous studies have shown that hyperlipidemic mice, after exposed to diesel exhaust or to ambient UFPs,[17,27] exhibited increased plasma levels of various hydroxyeicosatetraenoic (HETEs) and hydroxyoctadecadienoic acids (HODEs) as well as decreased PON-1 activity. HETEs and HODEs are oxidative products of polyunsaturated fatty acids (PUFAs), arachidonic and linoleic acids, respectively, which are generated at least partially via lipoxygenase (LOX) mediated oxidative pathways. The attempts to validate these biomarkers in humans failed because the study subjects were exposed to concentrated $PM_{2.5}$ or PM_{10} for only two hours, which yielded no effects on PON1 activity.[28,29] It is possible that longer exposure to PM/PAHs may be required to document significant effects.

1.5. Scope of work

The overarching goal of this work is to achieve a better understanding on the CV effects of PAHs in humans. This will be accomplished through a unique opportunity offered by the Joint Research

Institute (JRI, http://www.pku-jri.ucla.edu) between UCLA and Peking University (PKU) in Beijing, China. Each year, JRI selects about ~15 UCLA students to participate in a 10-week summer research exchange program in PKU. Given a more than 10-fold difference in airborne PAHs between Los Angeles (< 10 ng/m^3) and Beijing (>100 ng/m^3),[30,31] this summer program provides an unique natural experimental opportunity, which allows to evaluate in the same subject, the CV effects of a dramatic change in exposure to PAHs, between a relatively "clean" environment (UCLA) and a heavily polluted environment (PKU). The specific objectives include: (1) determining to what extent travel to a polluted environment leads to increased exposure to PAHs and lipid peroxidation; (2) testing a panel of biomarkers in humans to detect early CV effects in response to environmental exposures; and (3) characterizing the temporal trends of exposure to PAHs in Los Angeles and Beijing.

2. URINARY METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS AND THE ASSOCIATION WITH LIPID PEROXIDATION

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2.1. Abstract

Air pollution is among the top threats to human health in China. As air toxicants, PAHs could bring significant risks to population; however, the exposure to PAHs in China and its health impact are not fully understood. In 2012, a summer exchange program allowed 10 students travel from Los Angeles to Beijing and stay there for 10 weeks. Based on the program, this study investigated the difference in urinary concentration of 12 hydroxylated-PAHs (Σ_{12}OH-PAHs) and MDA between the two cities. The median concentration of Σ_{12}OH-PAHs in Beijing (14.1 μg g^{-1} creatinine) was significantly higher than that in Los Angeles (5.78 μg g^{-1} creatinine), indicating a higher exposure in Beijing. The ratios of homogenous OH-PAHs (e.g. 1-/2-OH-NAP) changed significantly between the two cities ($p<0.01$), which might suggest a potential alteration in metabolism subsequent to exposure. A significant association between Σ_{12}OH-PAHs and MDA ($p<0.01$) was observed, with the association varying between the two cities. This study suggests exposure to PAHs might be linked to metabolism alteration and calls for future studies to investigate the role this possible alteration played in the health effects of PAHs exposure.

2.2. Introduction

PAHs are a group of air pollutants that contain two or more fused aromatic rings. Their ubiquitous occurrence in the environment has raised increasing concerns due to their high emissions and significant toxicity. The global emission of PAHs was approximately 504 Gg in 2007, of which 21% was from China.[32] PAHs are mainly emitted from combustion sources, such as vehicle

emissions, household fuel consumption and tobacco smoke.[12] All of these sources are geographically close to densely populated areas and could therefore bring significant exposure and health risks. Humans are exposed to PAHs through various pathways including inhalation, ingestion, and dermal absorption.[12] To assess the total exposure to PAHs from different routes, urinary OH-PAHs, the metabolites of PAHs, are widely measured.[33]

PAHs are associated with various adverse health effects (e.g. micronuclei frequency, DNA damage, lung function, and heart rate variability),[19,34–36] and certain adverse health outcomes (e.g. lung cancer, cardiovascular diseases, birth defects, and diabetes).[15,37–39] The biological mechanism of these associations is not yet clear, and oxidative damage is suggested as a possible cause. It has been shown that reactive oxygen species (ROS) could be generated during the metabolism of PAHs. Then, ROS could attack biological molecules such as DNA, proteins and lipids, resulting in a series of health problems. MDA is a product of lipid oxidative damage and was widely used as a biomarker for lipid peroxidation.[40] MDA was previously found to be associated with both PAHs exposure and various diseases,[40,41] suggesting a potential role of lipid peroxidation between PAHs and the health effects.

In recent years, the severe air pollution in Beijing has created great concerns.[42] As toxic air pollutants, PAHs were also present in higher concentrations in Beijing than in other cities in the developed countries.[43] As shown in a previous study, repeated measurements on travelers could allow researchers to focus on the impacts of exposure with less interference from individual differences.[44] In 2012, a panel of 10 UCLA students traveled to Beijing and stayed for 10 weeks, providing an opportunity to study their PAH exposures and related lipid peroxidation. In this chapter, the first-morning urine samples of these students were collected before, during, and after the exchange program. Twelve urinary OH-PAHs and MDA were measured as surrogates for

exposure and lipid peroxidation, respectively. The aims of this chapter were as follows: (1) assess the exposure to PAHs in Beijing and Los Angeles; (2) characterize the differences in the ratios of OH-PAHs in the two cities to better understand their metabolism; and (3) investigate the association between OH-PAHs and MDA.

2.3. Method

Sample collection. All 10 subjects (four males and six females) in this study were healthy UCLA students. The age and body mass index (BMI) of the subjects at the time of sample collection were 23.3±5.8 (mean ±standard deviation; range: 20-39, same as below) years, and 21.1±1.4 (18.6-23.4) kg m^{-2}, respectively. All the subjects were self-reported nonsmokers and participated in the summer exchange program between UCLA (in Los Angeles) and PKU (in Beijing) in 2012. A total of 11 urine samples were collected for each subject before, during, and after the exchange program. Briefly, three urine samples were collected before the program (pre-LA, Jun. 7^{th} - Jun. 19^{th}) in Los Angeles. Five collections were conducted during the program (Beijing, Jun. 29^{th} - Aug. 8^{th}) in Beijing. The last three samples were collected after the program (post-LA, Oct. 8^{th} - Oct. 26^{th}) when the students returned to the Los Angeles. As PAHs are metabolized rapidly in animals and human, with half-lives of less than one day,[45,46] the urine collection begun at least one week after arrival in the new city to exclude the interference of previous PAH exposures in the former city. For each urine collection, the first morning urine after fasting for at least 8 hours was collected in polypropylene tubes and frozen at -20 ºC until analysis.

For each subject, a questionnaire was used to collection additional information for the 3 days prior to the sample collection. In the questionnaire, detailed information including cooking behaviors (cooking frequency, cooking fuel, and barbecuing), diet (the consumption of barbecue or baked meat), traffic-related activities (driving hours, public transportation usage, and time spent

near heavy traffic areas), and passive smoking, was collected. This study was performed in accordance with the guidelines and approval of the Institutional Review Boards of both UCLA and PKU, and informed consent was obtained from each subject.

Analytical method. A previously established method was used in this study to measure the urinary OH-PAHs.[40] Briefly, 2 mL of urine from each sample was spiked with ^{13}C-labeled 3-hydroxyphenanthrene (^{13}C-3-OH-PHE) as surrogate standards and adjusted to pH 5.5 with sodium acetate buffer. Next, the sample was added to 20 μL of β-glucuronidase/sulfatase (Helix Pomatia, Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37℃ overnight to hydrolyze conjugated phenols. The liquid-liquid extraction with hexane methyl tert-butyl ether mixture (9:1, v/v) was performed three times to extract the analytes. After blowing with nitrogen to near complete dryness, 0.1 mL of methanol and 1 mL of diazomethane solution were added to the extract, and the OH-PAHs were methylated at room temperature for 5 h. Next, the sample was cleaned with silica gel chromatography (0.6-cm i.d., 6-cm length, with 0.5-cm anhydrous Na_2SO_4 on top) and eluted with 8 mL of hexane, 8 mL of hexane dichloromethane mixture (3:2, v/v) and 8 mL of dichloromethane sequentially. The analytes were in the second and third fractions. Finally, the sample was concentrated, spiked with d_{10}-acenaphthene (d_{10}-ACE) and d_{10}-phenanthrene (d_{10}-PHE) as internal standards and analyzed using a gas chromatograph and mass spectrometer (GC/MS; Agilent 7890A-5975C) with an electron ionization ion source and a 30-m DB-5MS column (250-μm i.d., 0.25-μm film thickness; J&W Scientific, Folsom, CA, USA). The monitored ion couples for all analytes and the limit of quantification (LOQ) (ranged from 7.5 to 18.2 pg mL^{-1}) are listed in Table 2.1.

Urinary MDA concentrations were measured based on the reaction with 2-thiobarbituric acid (TBA). Briefly, a 150 μL urine sample mixed with 450 μL of TBA and 900 μL of phosphate

(0.5 mol L^{-1}) was incubated in water at 95 ℃ for 1 h. After being cooled and filtered, the mixture was injected into a high-performance liquid chromatograph (HPLC; Waters 2695) with a reverse-phase C18 column (150 mm in length, 3.9 mm i.d.) and a mobile phase of potassium phosphate (0.05 mol L^{-1}, pH=6.5) and methanol (60:40, v/v). The MDA-TBA adducts could be detected under a wavelength of 532 nm in a UV detector. The LOQ the method is 7.2 ng mL^{-1}. Urinary creatinine was measured by a spectrometer under a wavelength of 510 nm based on the Jaffe reaction.[47]

Table 2.1. Information of OH-PAHs standards, the monitored ions of methylated OH-PAHs in GC-MS and the LOQ of OH-PAHs

Compound	Abbreviation	Ions monitored	LOQ (pg mL^{-1})	Purity	Source
1-hydroxynaphthalene	1-OH-NAP	158, 159	14.9	98%	Dr. Ehrenstrofer[a]
2-hydroxynaphthalene	2-OH-NAP	158, 159	13.6	99%	Dr. Ehrenstrofer
2-hydroxybiphenyl	2-OH-BP	184, 185	13.0	97.7%	AccuStandard[b]
3-hydroxybiphenyl	3-OH-BP	184, 185	8.6	100%	AccuStandard
4-hydroxybiphenyl	4-OH-BP	184, 185	18.2	100%	AccuStandard
2,2'-dihydroxybiphenyl	2,2'-DOH-BP	214, 215	11.9	100%	AccuStandard
3,4-dihydroxybiphenyl	3,4-DOH-BP	214, 215	12.3	100%	AccuStandard
4,4'-dihydroxybiphenyl	4,4'-DOH-BP	214, 215	9.7	100%	AccuStandard
2-hydroxydibenzofuran	2-OH-DBF	198, 199	10.8	98.3%	Chiron AS[c]
2-hydroxyfluorene	2-OH-FLU	196, 197	7.5	98%	Aldrich[d]
3-hydroxyfluorene	3-OH-FLU	196, 197	12.3	NA[e]	Aldrich
1-hydroxyphenanthrene	1-OH-PHE	208, 209	8.8	99%	Dr. Ehrenstrofer
2-hydroxyphenanthrene	2-OH-PHE	208, 209	17.0	99%	Dr. Ehrenstrofer
3-hydroxyphenanthrene	3-OH-PHE	208, 209	12.8	99%	Dr. Ehrenstrofer
4-hydroxyphenanthrene	4-OH-PHE	208, 209	9.9	99.6%	Dr. Ehrenstrofer
1-hydroxypyrene	1-OH-PYR	217, 218	14.3	100%	AccuStandard

[a.] Augsburg, Germany; [b.] New Haven, CT, USA; [c.] Trondheim, Norway; [d.] St. Louis, MO, USA; [e.] not available.

Quality control. For each batch of eight urine samples, one laboratory blank sample (with 2 mL of purified water) was prepared. The analysis for blank samples was the same as that for urine samples. For all urine samples, three identical samples were prepared to ensure repeatability. The

concentrations of 3-hydroxybiphenyl (3-OH-BP, 15.8%), 2,2'-dihydroxybiphenyl (2,2'-DOH-BP, 12.2%), 3,4'-DOH-BP (14.5%), and 3-OH-PHE, 33.9% in the blank samples were more than 10.0% of the average concentrations in the urine samples and hence removed from the subsequent discussion. The concentrations of the remaining 12 analytes in blank samples were 1.11±1.05% of the average concentrations in urine samples. Thus, blank subtraction was not performed for all those analytes. The relative deviation of all analytes was 21.0 ± 7.2%. The recovery of ^{13}C-3-OH-PHE was 93.6 ± 12.4%. All the OH-PAHs and MDA data were normalized by creatinine.

Statistical analysis. The Shapiro-Wilk test was applied to check the normality of the data in this study. Median values (with interquartile range, IQR) were reported for urinary biomarkers and their ratios, unless otherwise noted. For analytes not detected in urine samples, the 1/2 LOQ was applied as a substitute for the statistical analysis. Mann–Whitney U-test was used to investigate the difference in urinary biomarkers and questionnaire data between the two cities. A two-tailed *p* value of <0.05 was considered significant. Multivariate linear regressions with step-wise or enter approaches were applied to identify the confounding factors and calculate the concentration ratios between the two cities. A simple linear regression model and three linear mixed-effects models were used to investigate the association between MDA and OH-PAHs. In the simple linear regression model (Model A), the association between OH-PAHs and MDA was considered constant among subjects in the two cities:

$$y_{ijk} = \alpha + \beta x_{ijk} + \varepsilon_{ijk} \quad [1]$$

where y_{ijk} and x_{ijk} are the log-transformed concentrations of MDA and OH-PAHs of subject *i* at time *j* in the city *k*; respectively. α and β is the fixed intercept and slope, respectively. ε_{ijk} is the residual. In the three mixed-effects models, a random intercept was allowed among subjects

(Model B, equation 2), cities (Model C, equation 3), and both subjects and cities (Model D, equation 4), respectively.

$$y_{ijk} = \alpha + \mu_i + \beta x_{ijk} + \varepsilon_{ijk} \quad [2]$$

$$y_{ijk} = \alpha + \mu_k + \beta x_{ijk} + \varepsilon_{ijk} \quad [3]$$

$$y_{ijk} = \alpha + \mu_i + \mu_k + \beta x_{ijk} + \varepsilon_{ijk} \quad [4]$$

where μ_i and μ_k is the random intercept for subject *i* and city *k*, respectively. All statistical analyses were conducted in SPSS package 18.0 (SPSS, Chicago, IL, USA).

2.4. Results and discussion

Concentrations of urinary OH-PAHs. For the 12 metabolites of PAHs in the subsequent discussion, the detection rates were all greater than 88%. The concentrations of OH-PAHs with different numbers of rings are shown in Figure 2.1. The median concentrations of hydroxynaphthalenes (ΣOH-NAPs, sum of 1- and 2-OH-NAP), ΣOH-BPs (sum of 2-, 4-OH-BP and 4,4'-DOH-BP), 2-hydroxydibenzofuran (2-OH-DBF), ΣOH-FLUs (sum of 2-, and 3-OH-FLU), ΣOH-PHEs (sum of 1-, 2-, and 4- OH-PHE) and 1-OH-PYR were 4.01, 2.12, 0.60, 0.56, 0.43 and 0.13 μg g^{-1} creatinine, respectively. A decreasing trend in urinary concentration of OH-PAHs was observed when the number of aromatic rings increased. This was likely because PAHs with fewer aromatic rings tend to present in a higher concentration in the environment[12] and have higher urine excretion rate in human body.[48,49]

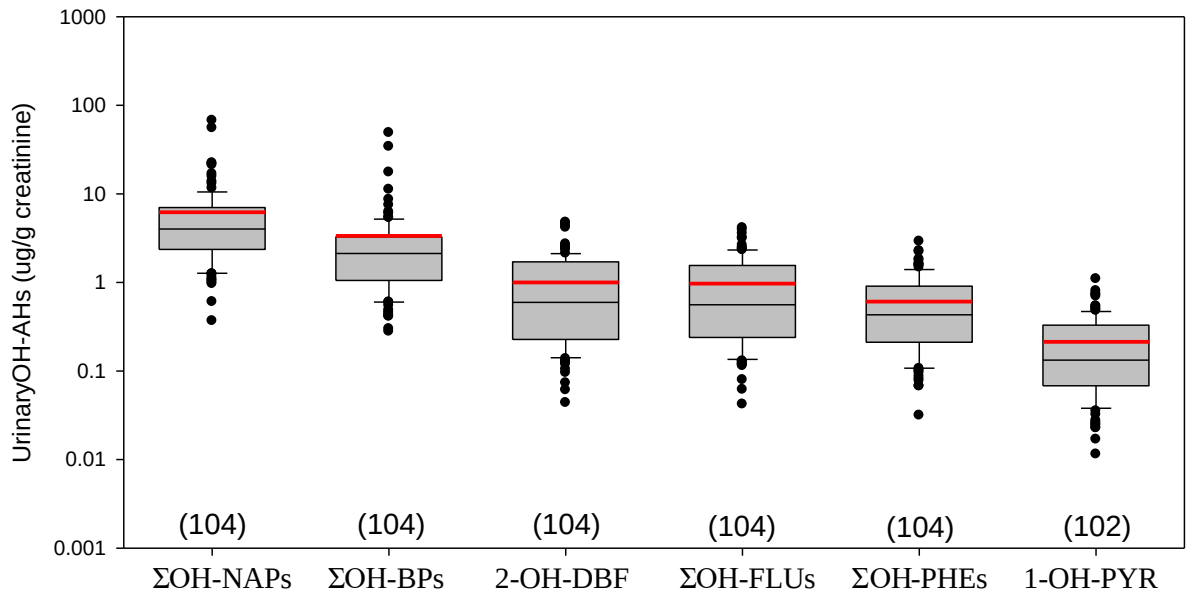

Figure 2.1. Concentration of grouped urinary OH-PAHs. The solid and red horizontal lines represent median and mean, respectively. The box and whiskers represent the 25th-75th and 10th-90th percentiles, respectively. Numbers in parentheses are the number of detections.

The concentration of urinary OH-PAHs was influenced by many factors, such as the environmental levels of PAHs, individual activity patterns, and individual characteristics. In this study, the determinants of OH-PAHs were investigated using a multivariate model with step-wise approach based on the questionnaire data. We found that city (i.e., Los Angeles and Beijing) was the dominant factor determining the urinary OH-PAHs concentrations. Individual characteristics (i.e., gender, age and BMI) were also significant factors (p<0.05); however, their impacts on the change of OH-PAHs between the two cities were minimized as multiple measurements were conducted for each subject who serves as his/her own control. Individual activity patterns, including diet habits and traffic-related activities, have limited impacts on the urinary OH-PAHs

concentrations in this study, because most of them were not significantly associated with OH-PAHs after adjustment for city. Thus, they were not considered in the subsequent discussion.

Table 2.2 shows the difference in the creatinine adjusted OH-PAHs concentration between Los Angles and Beijing. It should be noted that biphenyl and dibenzofuran are technically not PAHs but have similar structure and environment sources with PAHs. Hence in addition to the total concentration of all analytes (Σ_{12}OH-PAHs), the total concentration of metabolites of naphthalene, fluorene, phenanthrene and pyrene (Σ_{8}OH-PAHs) was also calculated (Table 1). The median concentration of Σ_{12}OH-PAHs in Beijing was 14.1 μg g^{-1} creatinine, which was significantly higher than that in pre- (5.77 μg g^{-1} creatinine, p<0.001) and post-LA (5.78 μg g^{-1} creatinine, p<0.001). No significant difference was observed between the Σ_{12}OH-PAHs concentration in pre-LA and post-LA (p=0.85). A similar trend was also observed for ΣOH-NAPs, ΣOH-BPs, 2-OH-DBF, ΣOH-FLUs, ΣOH-PHEs and 1-OH-PYR. These results indicate the observed urinary OH-PAHs levels were mainly driven by the differences of various environmental and activity factors between the two cities. Based on these biomarkers, it was estimated that the exposure to different PAHs was 1.3 - 6.1 folds higher in Beijing than in Los Angeles during the study season.

Table 2.2 Descriptive statistics of urinary OH-PAHs in Beijing and Los Angeles before (pre-LA) and after the trip (post-LA)

Biomarker	Median (IQR)			*p*-value [a]			Beijing/Los Angeles concentration Ratio (95%CI; *p*-value) [b]
	pre-LA(n=30)	Beijing(n=47)	post-LA (n=27)	pre-LA vs. Beijing	post-LA vs. Beijing	Pre- vs. post-LA	
∑OH-NAPs	3.08 (1.85, 5.82)	5.01 (2.95, 7.94)	2.76 (1.87, 4.41)	<0.05	<0.05	0.57	1.3 (0.97-1.8; 0.08)
∑OH-BPs	1.39 (0.72, 2.22)	2.93 (1.84, 4.79)	1.54 (0.94, 2.77)	<0.001	<0.01	0.33	2.3 (1.6-3.2; <0.001)
2-OH-DBF	0.25 (0.14, 0.47)	1.80 (1.24, 2.07)	0.25 (0.20, 0.34)	<0.001	<0.001	0.60	6.1 (4.6-7.9; <0.001)
∑OH-FLUs	0.29 (0.13, 0.49)	1.58 (1.17, 2.31)	0.26 (0.17, 0.34)	<0.001	<0.001	0.91	5.6 (4.3-7.4; <0.001)
∑OH-PHEs	0.25 (0.15, 0.43)	0.92 (0.52, 1.26)	0.20 (0.13, 0.36)	<0.001	<0.001	0.48	3.5 (2.6-4.6; <0.001)
1-OH-PYR	0.09 (0.05, 0.16)	0.32 (0.18, 0.46)	0.07 (0.05, 0.13)	<0.001	<0.001	0.99	3.3 (2.4-4.6; <0.001)
$\sum_8$OH-PAHs	3.76 (2.16, 7.06)	8.85 (4.99, 12.1)	3.27 (2.37, 5.54)	<0.01	<0.001	0.62	1.8 (1.3-2.4; <0.001)
$\sum_{12}$OH-PAHs	5.77 (3.63, 10.6)	14.1 (7.68, 20.5)	5.78 (3.70, 10.5)	<0.001	<0.001	0.85	2.0 (1.5-2.7; <0.001)

a. Mann-Whitney test

b. Ratio=10^{β}, where β is the estimated slope for city in multivariate linear regression models with enter approach, where OH-PAHs is log-transformed. Ratios are adjusted by age, gender and BMI

As a classic biomarker for PAHs exposure, 1-OH-PYR is widely measured in populations around the world.[33] Hence, it was used for comparison with other studies. As shown in Figure 2.2, the concentration of 1-OH-PYR in Beijing (median, 0.32 μg g^{-1} creatinine) was higher than that of most cities in developed countries, such as San Francisco, USA (0.08 μg g^{-1} creatinine)[46] and Christchurch, New Zealand (0.04 μg g^{-1} creatinine),[50] but lower than that of most cities in developing countries, such as Nanjing, China (1.08 μg g^{-1} creatinine)[51] and Bangkok, Thailand (0.39 μg g^{-1} creatinine).[52] The concentration of 1-OH-PYR in Los Angeles (0.08 μg g^{-1} creatinine) was comparable to that in cities in developed countries. Those comparisons indicated that the exposure to PAHs in the summer in both Beijing and Los Angeles was at an intermediate level worldwide.

Difference in the ratios of OH-PAH isomers. As discussed above, ΣOH-NAPs and ΣOH-BPs differed significantly in the two cities; however, not all metabolites from the same precursor PAHs showed the same concentration ratios between the two cities. Briefly, the concentration ratios of 1-OH-NAP, 4-OH-BP, and 4,4'-DOH-BP between Beijing and Los Angeles were significantly greater than 1.0 (p<0.001). In contrast, the concentration ratios of 2-OH-NAP and 2-OH-BP were not significantly different with 1.0. The difference of these OH-PAH isomers indicated potential bias may exist if only one or few isomers were used as surrogates for total PAHs exposures. Instead, the sum of OH-PAHs isomers (i.e. ΣOH-NAPs and ΣOH-BPs) could be the least-biased surrogate for PAHs exposure given the concentrations of multiple OH-PAHs are available.

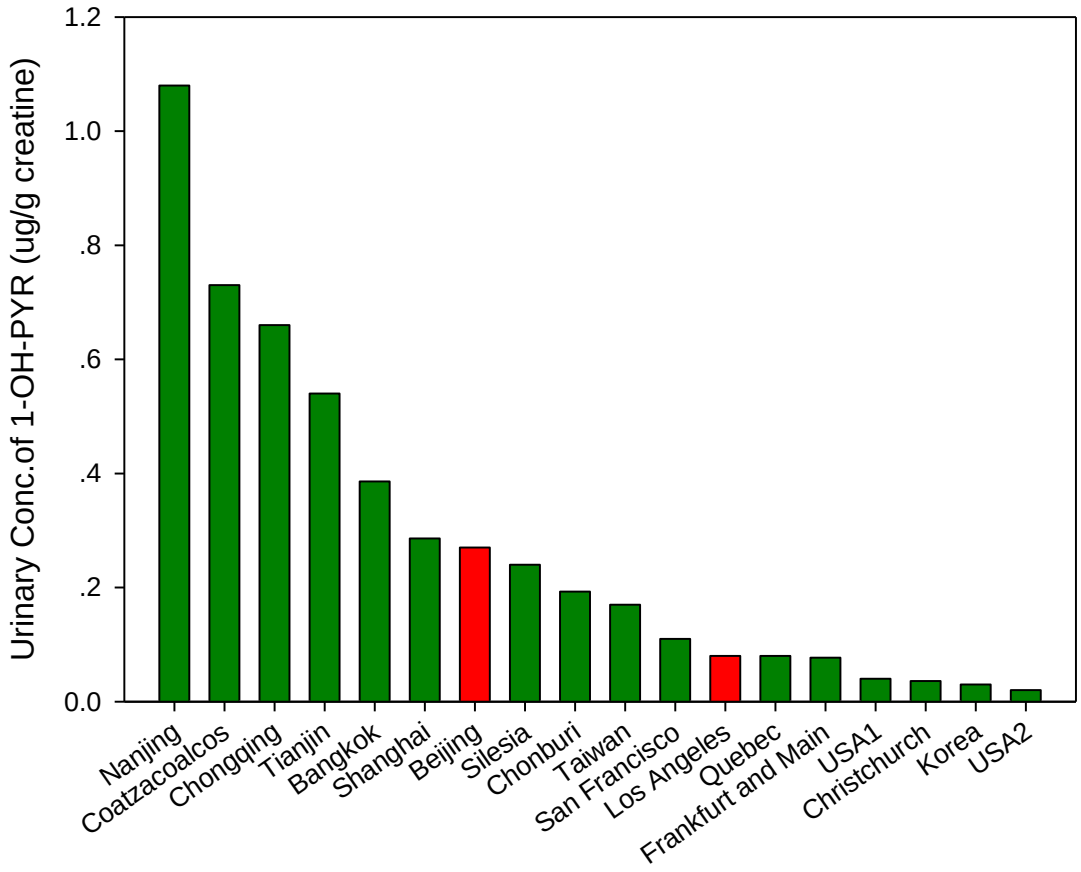

Figure 2.2. Concentration of urinary 1-OH-PYR in the population around world. Red bars are results from this study. Nanjing: 273 male adults;[51] Coatzacoalcos: 82 school children;[53] Chongqing: 343 male adult;[54] Tianjin: 116 adults in rural sites;[40] Bangkok: 105 schoolchildren;[52] Shanghai: 343 female adults;[55] Silesia: 108 non-smokers;[46] Chonburi: 61 schoolchildren;[52] Taiwan: 93 indoor officers;[56] San Francisco: 76 non-smokers;[46] Quebec:140 adults;[57] Frankfurt and Main: 289 non-smokers with age>20;[57] USA1: 1309 people with age>20; [58] Korea: 129 male university students;[59] Christchurch: 89 male students;[50] USA2: 1625 people with age>20.[60]

The reason for the different concentration ratios of OH-PAH isomers between the two cities is unclear, and the interaction between PAHs and cytochrome P450 (CYP) enzymes may be a possible mechanism. PAHs could be metabolized by a series of CYP enzymes, such as CYP1A1 and CYP1B1, through an arene oxide intermediate to form hydroxylated metabolites.[61] Different CYPs in the phase I metabolism of PAHs could result in different metabolite (i.e., OH-PAHs) ratios.[62,63] Meanwhile, PAHs and their metabolites could in turn induce or inhibit the expression of CYPs,[64] which could alter the profiles of CYP enzymes involved in the metabolism of PAHs, and then further alter the ratios of OH-PAHs isomers. In this study, a higher exposure to PAHs was observed in Beijing, which could possibly cause a shift in the relative expression of different CYPs and might therefore lead to a corresponding shift in the OH-PAHs isomer ratios.

Since previous studies revealed a difference in PAHs metabolite ratios under the catalysis of different CYPs,[62,63] we suspect there may be a link between the alteration of OH-PAHs isomer ratios and the exposure-induced alteration of CYPs expression. To test this hypothesis, we investigated the difference in several OH-PAHs isomer ratios between the two cities. First, the ratio of 1-OH-NAP to 2-OH-NAP (1-/2-OH-NAP) was investigated because (1) 1-OH-NAP and 2-OH-NAP were the only monohydroxylated metabolites of naphthalene so that the ratio would not be influenced by other monohydroxylated metabolites; and (2) the 1-/2-OH-NAP was mathematically independent from the ΣOH-NAPs. As expected, the 1-/2-OH-NAP ratio was significantly elevated in Beijing, suggesting a possible shift in the relative expression of CYPs. It should be noted that the elevation of 1-/2-OH-NAP occurred gradually after the students arrived in Beijing (Figure 2.3), possibly suggesting the alteration of metabolism could be a subacute process. This may explain the observation in other studies that the variation of OH-PAHs isomer concentrations tended to be more consistent after an accidental high exposure.

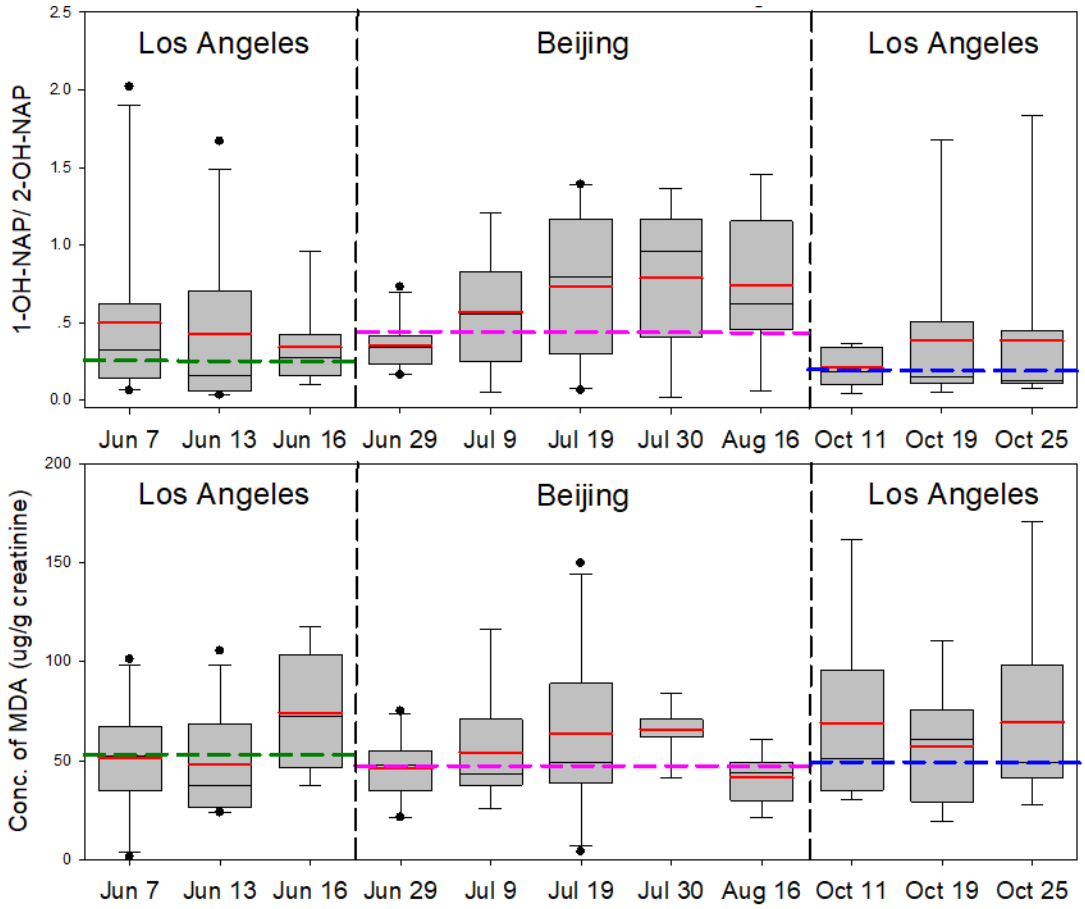

Figure 2.3. Temporal trend of 1-OH-NAP/2-OH-NAP and MDA in urines. The solid and red horizontal lines represent median and mean, respectively. The box and whiskers represent the 25th-75th and 10th-90th percentiles, respectively.

It should be noted that 1-OH-NAP is also a metabolite of carbaryl pesticides.[65] If 1-/2-OH-NAP was influenced by carbaryl pesticides exposure, we would expect that 1-/2-OH-NAP had a more significant association with ΣOH-NAPs than with other OH-PAHs. However, 1-/2-OH-NAP was not correlated with ΣOH-NAPs, but significantly correlated with other OH-PAHs, indicating carbaryl pesticides have limited impacts in this study.

To further confirm the relationship between the 1-/2-OH-NAP ratio and PAHs exposure, an analysis was conducted on selected literatures. The selection criteria include: (1) the population was under a well-defined long-term exposure; (2) the PAHs to which the population was exposed were mainly from combustion sources to minimize the interference from carbaryl pesticide; and (3) the sample size is larger than 10 to decrease the uncertainty caused by individual difference. As the number and species of the OH-PAHs measured varied among different studies, the concentration of ΣOH-NAPs was used as an indicator for total exposure to PAHs. The results are shown in Figure 2.4. A significant association was observed between the 1-/2-OH-NAP and ΣOH-NAPs (R^2=0.52, p<0.001). Additionally, for studies in which repeated measurements were conducted that minimized the genetic factors, an inter-study relationship between 1-/2-OH-NAP and ΣOH-NAPs was also observed.[66–71] These results revealed a potential shift in the relative expression of CYPs that might be related to PAH exposures.

The alteration of 1-/2-OH-NAP could explain why 1-OH-NAP and 2-OH-NAP had different concentration ratios between the two cities. For 1-OH-NAP, the exposure to PAHs and the corresponding alteration of 1-/2-OH-NAP were in the same direction; therefore, the concentration of 1-OH-NAP was significantly higher in Beijing. However, for 2-OH-NAP, the change in exposure to PAHs could be offset by alterations in the ratio; thus, the concentration of 2-OH-NAP was observed to be similar in the two cities.

This mechanism could also explain the observation of OH-PHEs isomers. Previous studies have shown that 1-OH-PHE and 2-OH-PHE are mainly derived from the same CYPs (e.g. CYP1A1), while 3-OH-PHE and 4-OH-PHE from other CYPs (e.g. CYP1A2).[62] These findings were consistent with the observations in this study that the 1+2-/4-OH-PHE ratio was significantly elevated in Beijing (p<0.01). In addition, 1+2-/4-OH-PHE was significantly correlated with

several OH-PAHs (i.e. 2-OH-DBF and ΣOH-FLU, p<0.05), possibly suggesting a similar link between exposure and metabolism.

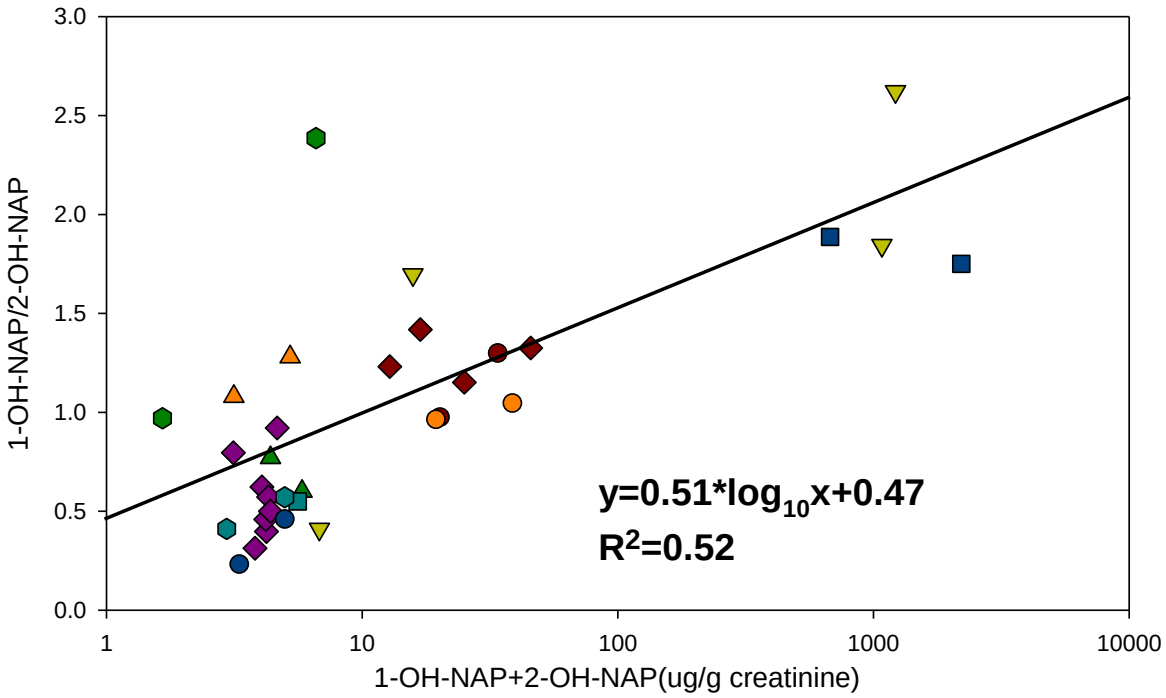

Figure 2.4. The association between naphthalene exposure and 1-/2-OH-NAP ratio.(●- cooking women;[69] ● - cooking women;[68] ▼- coking workers;[71] ▲ - road construction workers;[70] ■ - healthy general people;[51] ■- coking workers;[72] ◆- general people near an aluminum plant;[67] ◆- Coking workers;[19] ▲- general people near a creosote impregnation plant;[73] ▼- schoolchildren near a road;[74] ⬢- brick kiln workers;[66] ⬢- U.S. air forces personnel;[66] ⬢- this study.)

Previous studies found that smoking could decrease 1+2-/3+4-OH-PHE, suggesting exposure to secondhand smoke (SHS) may reduce the 1+2-/4-OH-PHE.[46] In our study, self-reported SHS exposure is significantly higher in Beijing (p<0.05). To distinguish the impacts of PAHs exposure from SHS and non-SHS sources, we divided the data in Beijing into two groups. As shown in Figure 2.5, all subjects in Beijing had significantly higher ΣOH-PHEs and 1+2-/4-

OH-PHE than in Los Angeles. Subjects in Beijing with SHS exposures tend to have slightly higher ΣOH-PHEs but lower 1+2-/4-OH-PHE compared with those without SHS exposures, which was consistent with previous studies on smoking. However, no significant difference was observed between subjects with and without SHS exposures in Beijing. These results indicate that the elevation of ΣOH-PHEs and 1+2-/4-OH-PHE in Beijing was probably attributed to sources other than SHS.

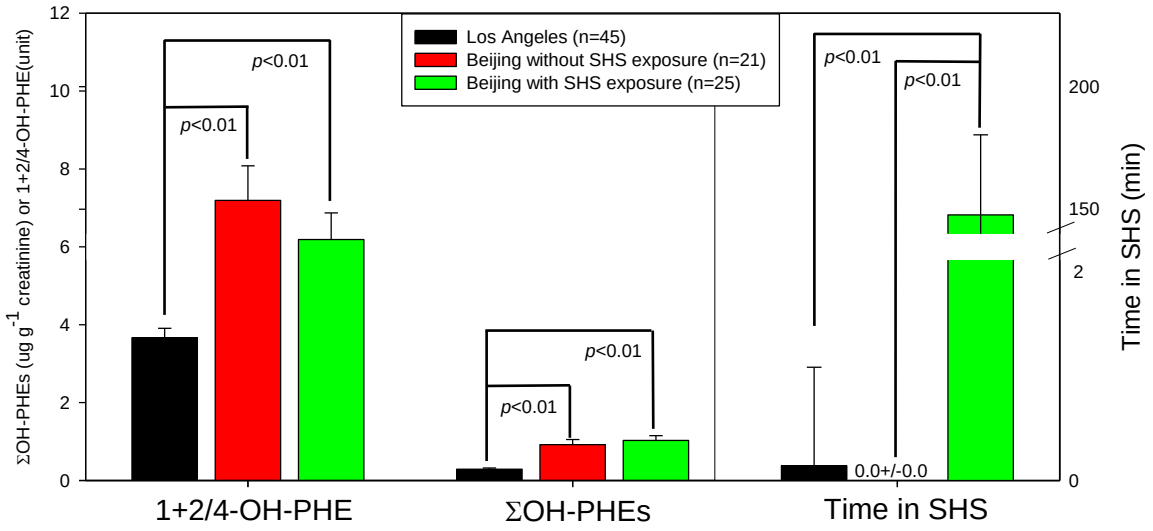

Figure 2.5. Comparison in 1+2/4-PHEs, ∑OH-PHEs and time in secondhand smokes (SHS) among population in Los Angeles, in Beijing without SHS exposure and in Beijing with SHS. Error bar indicates the standard error.

Association between MDA and OH-PAHs. MDA was a product of lipid oxidative damage and hence was used as an indicator of lipid peroxidation.[40,41] In this study, MDA was detected in all the urine samples and their median concentrations were 48.4 and 51.9 μg g^{-1} creatinine in Beijing and Los Angeles, respectively. No significant difference in the concentration of MDA was observed between the two cities (Figure 2.3). The relationship between MDA and OH-PAHs is

shown in Figure 2.6. MDA was significantly correlated with Σ_{12}OH-PAHs (p<0.05), however, for speciation analysis, only ΣOH-BPs was significantly correlated with MDA (p<0.05). This result is out of our expectation because most species measured in this study were found to strongly associate with MDA or other oxidative damage biomarkers (i.e. 8-hydroxy-2-deoxyguanosine and 8-isoprostane) as shown in previous studies.[19,40] In addition, the association between MDA and several OH-PAHs is marginally significant (Figure 2.6), suggesting there are some interference factors affecting the association.

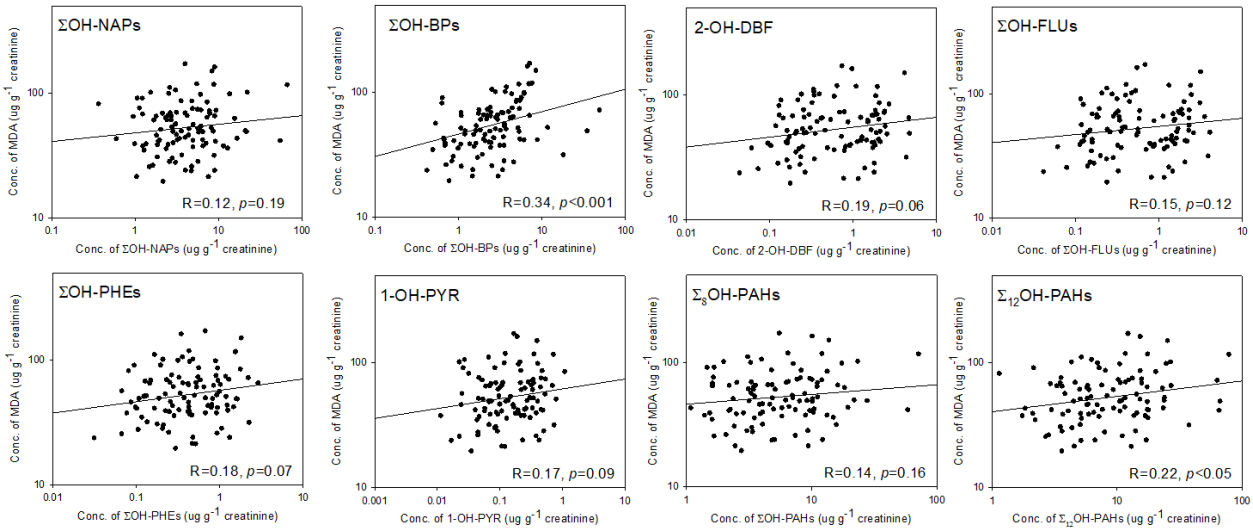

Figure 2.6. Correlation between MDA and OH-PAHs.

To investigate the possible interference factors, we applied a simple linear regression model (Model A) and three mixed-effects models (Model B, C and D) to study the association between MDA and OH-PAHs and then compared the results of different models. In Model A, the association between OH-PAHs and MDA was considered constant among individuals and cities, which is corresponding to the results in Figure 2.6. Among six OH-PAHs homologues, only ∑OH-BPs is significantly associated with MDA (p<0.05). In Model B, the intercept could vary among subjects. As shown in Figure 2.7, the association between OH-PAHs and MDA was comparable

with that in Model A, indicating that individual difference didn't cause a significant interference in this study. In Model C, the intercept could vary between the two cities, and the results revealed significant association between MDA and all OH-PAHs except for ∑OH-NAPs. Compared with Model A, the association between OH-PAHs and MDA was generally more significant, indicating city is a major interference factor. The results of Model D, in which the intercept was varied among both subjects and cities, were similar with that of Model C, once again indicating a limited impact of individual difference compared with city.

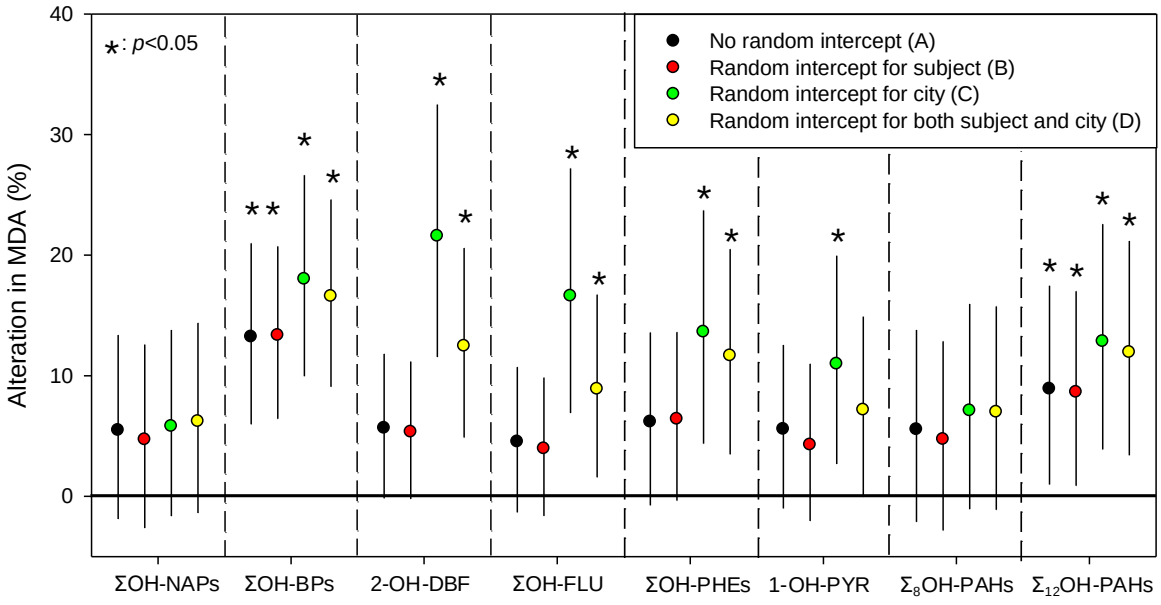

Figure 2.7. Associations between OH-PAHs and MDA. The alteration in MDA (%) was associated with one-folder increase of OH-PAHs.

As discussed above, the association between OH-PAHs and MDA was found to vary between the two cities, even for the same subject. There are several possible explanations for the observed city-effect on associations: (1) the exposure to PAHs could induce the change in antioxidants in the human body, which could affect an individual's oxidative stress;[75] (2) the urinary MDA concentration was affected by other factors differing in two cities, such as the diet

intake of MDA precursors and the decomposition conditions of MDA;[10] and (3) the cities' differences in the concentration of other pollutants that could induce oxidative damage may interfere with the association between MDA and OH-PAHs.[18] However, the potential mechanism of the observed city-effect is beyond the scope of this study and future studies are warranted. Even so, it is important to address that the associations between MDA and OH-PAHs are generally significant only if city-effect was considered. This is probably because OH-PAHs were significantly higher in Beijing, but MDA was comparable between the two cities, which could weaken the significant within-city associations.

There are several limitations of the study in this chapter. First, the external exposures to PAHs were not measured, thus the OH-PAHs results could not be attributed to specific sources. For example, the time spent in indoor environments was not assessed in this study but may be an important factor affecting PAHs exposure levels and the related health effects.[18] Second, many factors (e.g., diet, stress, and physical activities, etc.) may have changed when the subjects traveled from Los Angeles to Beijing. How these factors affect OH-PAHs measured in this study is not fully understood. Finally, since CYP enzyme can't be readily measured in human subjects, how it affects the observed difference of the ratios of some OH-PAH isomers between the two cities can't be determined.

In this chapter, we identified significantly higher PAHs exposure and homogenous OH-PAHs ratios in Beijing compared with Los Angeles in summer 2012. We also found a significant association between PAHs exposure and lipid peroxidation, with the association varying between the two cities. This study highlighted a possible link between PAH exposure and metabolism which needs to be considered in future health effect studies.

3. EARLY CARDIOVASCULAR EFFECTS AFTER TRAVELING FROM LOS ANGELES TO BEIJING

3.1. Abstract

Long-term exposure to PM is associated with increased CV mortality. Prevention of PM-induced chronic CV diseases depend on our ability to detect early effects, which is limited by the lack of sensitive biomarkers. We have identified promising biomarkers in animal models but comparable evidence in humans is lacking. In this chapter, we aim to fill this gap by following 26 non-smoking healthy young adults who traveled from Los Angeles to Beijing and collected paired blood and urine samples before, during, and after the trip. We assessed a panel of circulating biomarkers indicative of lipid peroxidation and inflammation. Personal exposure to PAHs was assessed by urinary PAHs metabolites. Traveling from Los Angeles to Beijing induced lipid peroxidation likely due to lipoxygenase activation as indicated by increased levels of 5-, 12-, 15- HETEs and 9-, 13-HODEs (False discovery rate, FDR<0.05). It also led to decreased anti-oxidative paraoxonase and arylesterase enzymatic activities (FDR<0.05) and increased pro-inflammatory fibrinogen concentration (FDR<0.05). Likewise, urinary PAHs metabolites were elevated in Beijing (FDR<0.05) and associated with CV biomarkers (p<0.05). All changes in measured biomarkers reversed, at least partially, after subjects returned to Los Angeles. These results indicated that HETEs, HODEs, paraoxonase and arylesterase activities have potentials to serve as sensitive biomarkers to identify early CV effects induced by PM. Substantively, our results suggest that people might be at higher CV risk when traveling to a more-polluted city.

3.2. Introduction

Long-term exposure to ambient particulate matter (PM) was estimated to cause 2.4 million world-wide premature deaths due to CV and cerebrovascular diseases,[76] a majority of which was of

ischemic nature.[16] PM pollution is a serious public health problem in populous developing countries such as China, which adversely affects the CV health of not only 1.4 billion Chinese residents [42] but also 60 million international visitors every year.[3] Epidemiological studies have associated exposure to PM with atherosclerosis,[5] and animal studies support these associations as causal.[10] Our ability to detect early health effects of PM before development of atherosclerosis and chronic CV diseases is crucial for the prevention of clinical CV events but this is limited by the lack of sensitive biomarkers.

Substantial evidence suggests that systemic oxidation and inflammation are central to PM's ability to promote atherosclerosis.[77] PM's redox activity may be due, at least partially, to combustion-originated components such as PAHs. [78] This is supported by cross-sectional studies showing associations between long-term exposure to PM or PAHs and oxidative biomarkers such as 8-isoprostane in the blood.[22] In controlled experimental and panel studies, however, the effects of short-term exposure to PM on oxidative biomarkers are inconsistent.[22] The lack of significant acute or subacute effects in those studies might be due to the exposure duration or dose, timing of sample collections, suboptimal sensitivity of the biomarkers employed, or some combination of these factors. Therefore, to detect early CV effects, it is important to identify new biomarkers that are sensitive and informative on pathogenetic mechanisms.

LOX and PON1 are pro- and anti-oxidative enzymes, respectively, which are thought to play important roles in the pathogenesis of atherosclerosis in both animals[23,24] and humans [25,26], making their assessment attractive in the evaluation of PM-induced CV effects. In our laboratory's previous animal studies, we have demonstrated that two-week exposure to diesel exhaust or ten-week exposure to ambient ultrafine particles led to increased plasma levels of oxidative products of polyunsaturated fatty acids (PUFAs) such as HETEs and HODEs, likely due to activation of various LOXs, and inhibited PON1 activity.[17,27] Comparable evidence in humans, however, is

lacking. Thus, we found no effects in heathy adults from short-term (2-hour) exposure to $PM_{2.5}$ or coarse PM ($PM_{2.5-10}$) on PON1 activity.[28,29] It is possible that longer exposure to PM may be required to document the effects of PM on PON1.

The summer exchange program between UCLA and PKU provided a natural experimental opportunity to study the CV effects induced by subacute exposure to air pollutants in humans. In this chapter, we tested the hypothesis that traveling from less-polluted Los Angeles to more-polluted Beijing would lead to higher exposure to PAHs, induction of lipid peroxidation via LO activation, and perturbation of PON1 activity.

3.3. Method

Subjects and study design. Subjects were recruited from the UCLA students who visited PKU in the summers of 2014 and 2015. Inclusion criteria were age >18 years and body mass index (BMI) < 30 kg/m^2. Exclusion criteria included history of smoking, heart disease, metabolic disorders or having symptoms of asthma, kidney disease, blood coagulation disorders, rheumatological diseases or chronic inflammation during the previous six months. The study was approved by the institutional review boards at UCLA and PKU.

Beijing served as a natural exposure chamber due to its severely high air pollution levels. As illustrated in Figure 3.1, we collected three pairs of morning urine and blood samples from each subject, in Los Angeles (pre-LA, 1 – 3 weeks before departure), Beijing (6 – 8 weeks after arrival) and in Los Angeles again (post-LA, 4 – 7 weeks after returning). Subjects were asked to fast for 8 hours before sample collection to diminish dietary effects.

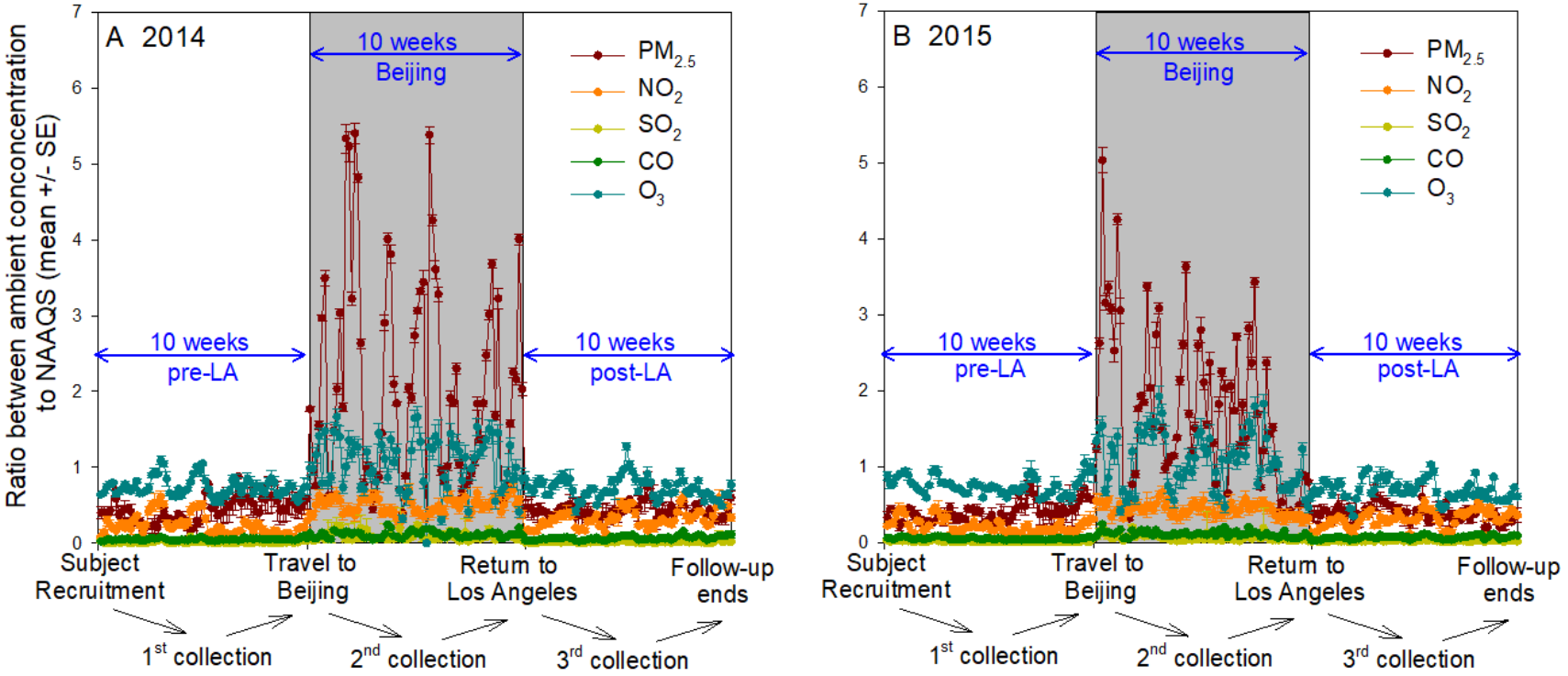

Figure 3.1. Schematic study design and temporal trend of criteria air pollutants in 2014 (Panel A) and 2015 (Panel B). Air pollution data in Los Angeles and Beijing were obtained from national air quality monitors within 30 km of UCLA (n=4) and Peking University (n=18), respectively; NAAQS-National Ambient Air Quality Standards: $PM_{2.5}$ - 35 µg/m^3 (daily average); NO_2 – 100 ppb (1-hour daily maximum); SO_2 – 75 ppb (1-hour daily maximum); CO – 9 ppm (8-hour daily maximum); O_3 – 70 ppb (8-hour daily maximum)

Laboratory analysis. As primary outcomes of the study, 13 circulating biomarkers were measured. Briefly, HETEs and 8-isoprostane are oxidative products of arachidonic acid through LOX-mediated and non-enzymatic pathways, respectively.[24] HODEs acids are oxidative products of linoleic acids from miscellaneous sources, including 12/15-LOX and non-enzymatic pathways.[79] We modified a previously established method to measure the free arachidonic and linoleic acids, and their oxidized products (i.e. HETEs and HODEs) in plasma.[17] Briefly, 150 µL plasma samples were spiked with d_8-15-HETE internal standard and the pH of the samples was adjusted to ~ 3.0 using 250 µL of 50% acetic acid. After equilibration for 15 min, the sample was loaded onto a preconditioned 1cc Oasis HLB solid-phase extraction cartridge (Waters) that had been previously been equilibrated with 1 ml methanol followed by 1 ml water before the sample loading. The sample was slowly loaded on the cartridge, and the cartridge was washed with 1mL 5% methanol in water. The analytes were eluted with 1 ml methanol. The eluate was then evaporated to dryness under a nitrogen stream. Methanol (50 uL) was added to the dried extract, vortexed for 30s, and the reconstituted extract was centrifuged at 4,000 rpm for 20 min at 4 Celsius and then quantified using an HPLC/Ion trap-Orbitrap-MS (Thermo Scientific). The analyte peak areas were corrected by the internal standard peak area. The recovery of HETEs and HODEs ranged from 100.9 to 107.9%, while that of arachidonic and linoleic acid was only 50.0% and 57.3%, respectively. The concentrations of arachidonic and linoleic acid were adjusted by the method recoveries. Blank samples were prepared for each 15 plasma samples, and negligible contamination was observed for all analytes.

PON1 enzymatic activity was assessed by the hydrolysis rate of diethy-*p*-nitrophenyl phosphate (paraoxonase)[80] or phenyl acetate (arylesterase).[81] Paraoxonase activity was determined as the rate of hydrolysis of paraoxon substrate (diethyl-*p*-nitro-phenyl phosphate) to *p*-nitrophenol.[80] Briefly, 5 µl of plasma was incubated with 2.4 mM of paraoxon substrate in 0.1 M

Tris–HCl buffer (pH 8.5) containing 2 mM $CaCl_2$ and 2.0 M NaCl at room temperature. The kinetics of *p*-nitrophenol formation was determined by recording absorbance at 405 nm every 15 s for 4 min. Arylesterase activity was determined as the rate of hydrolysis of phenyl acetate to phenol.[81] Briefly, 4 μl of plasma was incubated with 3.5 mM of phenyl acetate in 9 mM Tris–HCl buffer (pH 8.0) containing 0.9 mM $CaCl_2$ at room temperature. The kinetics of phenol formation was determined by recording absorbance at 270 nm every 15 s for 2 min. Both activities were expressed as nmol of product formed per minute for 1 mL of plasma.

Plasma free 8-isoprotane concentration was measured with a commercially available enzyme-linked immunosorbent assay (ELISA, Cayman Chemical).[17] Serum CRP, fibrinogen and von Willebrand factor (vWF) concentrations were measured with a commercial Millipore MILLIPLEX human cytokine/chemokine kit (Millipore Sigma). Plasma total and HDL cholesterol concentrations were assessed using the Infinity Cholesterol Reagent (Thermo Scientific).[17] In addition to the primary outcomes, we also explored the plasma concentrations of arachidonic and linoleic acids with LC/MS, and PON1 protein with an ELISA kit (BosterBio).

Exposure to PAHs was assessed by urinary monohydroxylated metabolites of PAHs as described in Chapter 2. We used GC/MS with an isotope dilution method to measure the concentration of urinary cotinine. Deuterium-labeled (+/-)-cotinine internal standard was added into 0.5 mL urine aliquots that were then treated with 0.5 mL of aqueous NaOH (4M)/KCl (1M). Then the samples were extracted with 2 mL dichloromethane four times, and the pooled organic extracts were concentrated under a nitrogen stream prior to analysis. Blank samples were prepared for each of the eight urine samples, and cotinine was undetected in all blank samples. Both OH-PAHs and cotinine concentrations were normalized by urinary creatinine concentrations.

Statistical analysis. All reported biomarkers were detected in more than 75% of the samples with undetected biomarkers assigned with a concentration of 50% of the LOQ. For exposure and CV biomarkers in each phase (i.e. pre-LA, Beijing, and post-LA), the mean (+/- standard deviation) or geometric mean with the interquartile range was tabulated, as appropriate. The difference in biomarker concentrations between phases was evaluated in linear mixed effects models with random effects at the subject level. The intra-individual correlation coefficient (ICC) of different biomarkers was estimated in the same model. Multiple comparisons among exposure (n=6) and CV biomarkers (n=13) were adjusted by the Benjamini-Hochberg method to control the overall FDR of 5%. The associations between biomarkers were examined in linear mixed effects models with random effects at both subject and phase levels. Significance was established with $p<0.05$ and FDR<0.05 when applicable.

3.4. Results

Twenty-seven students in the UCLA/PKU summer exchange program in 2014 and 2015 gave consent to participate in the study. We excluded one subject due to hypertension. The 26 subjects included 12 males and 14 females, with an average age of 23.8 ± 5.6 years (mean ± standard deviation) and BMI of 21.6 ± 2.4 kg/m^2. Twenty subjects completed all three sample collections, while six subjects completed two collections, missing one collection in either pre-LA, Beijing, or post-LA.

The concentration of ambient $PM_{2.5}$ in Beijing was 4.6-fold higher than that in Los Angeles, exceeding U.S. National Ambient Air Quality Standards (NAAQS) of 35 μg/m^3 in 69.4% of the days in the studied period (Figure 3.1). Other criteria air pollutants were also at higher levels in Beijing as shown in Figure 3.1. There were significant increases in the urinary concentrations of 1-OH-PYR (2.8-fold, Beijing/pre-LA), ΣOH-PHEs (3.8-fold), ΣOH-FLUs (6.7-fold), and 2-OH-

DBF (9.0-fold) as well (FDR<0.05), but not ΣOH-NAPs. The elevation of urinary OH-PAHs reversed in post-LA, but their levels were still significantly higher than those in pre-LA (FDR<0.05, Figure 3.2). Similarly, urinary cotinine, an indicator of passive smoking, was significantly higher in Beijing than pre- or post-LA (FDR<0.05). The differences in 1-OH-PYR, ΣOH-PHEs, ΣOH-FLUs, and 2-OH-DBF between Beijing and Los Angeles (both pre-LA and post-LA) were smaller but remained significant after adjusting for urinary cotinine (p<0.05, Figure 3.3), indicating that air pollution, among other sources, significantly contributed to increased PAHs exposure in Beijing.

There was a remarkable increase in the concentrations of 5-, 12- and 15-HETEs, as well as 9- and 13-HODEs in Beijing as compared with baseline levels in pre-LA (FDR<0.05), which at least partially reversed in post-LA (Figure 3.4 and Table 3.1). In comparison, an increase in 8-isoprostane was also observed in Beijing but did not reach statistical significance (Figure 3.4 and Table 3.1). The levels of HETEs and HODEs were significantly correlated with their parental compounds, arachidonic and linoleic acid, respectively (p<0.05) (Figure 3.5). While the levels of arachidonic acid remained unchanged, we observed a significant increase in the levels of linoleic acid in Beijing as compared with pre-LA (p<0.01). Normalization of the levels of HETEs by arachidonic acid resulted in higher ratios of 5-, 12-, and 15-HETEs to arachidonic acid in Beijing. In contrast, normalization of the levels of HODEs by linoleic acid resulted in comparable ratios of 9-, and 13-HODEs to linoleic acid among pre-LA, Beijing and post-LA.

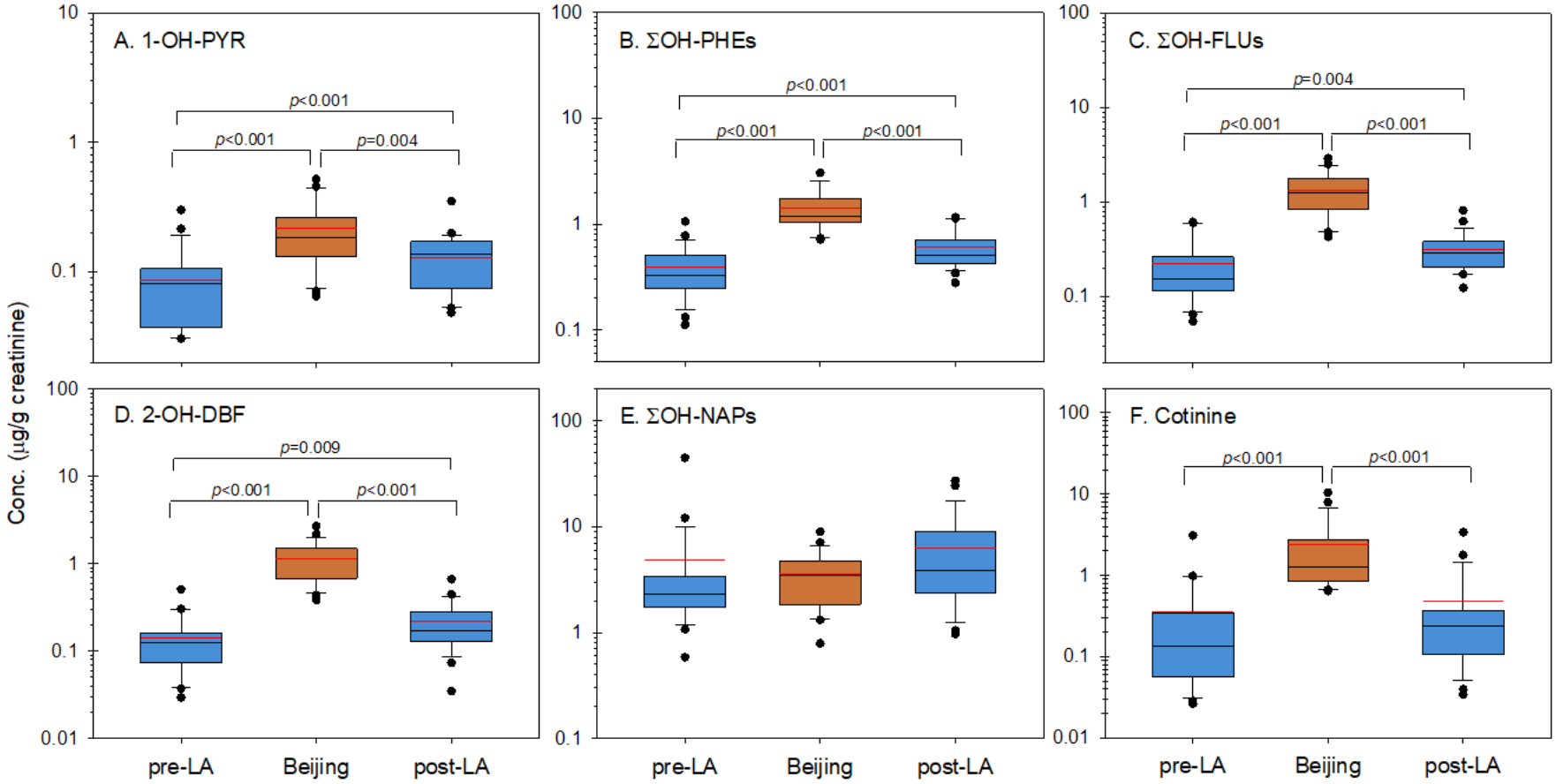

Figure 3.2. Urinary concentrations of OH-PAHs (Panels A-E) and cotinine (Panel F) in pre-LA, Beijing, and post-LA. All the *p*-values indicate FDR<0.05 after Benjamini-Hochberg adjustment; blue and orange boxes indicate data from Los Angeles and Beijing samples, respectively. The solid horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25th-75th percentiles, and the whiskers represent the 10th and 90th percentiles.)

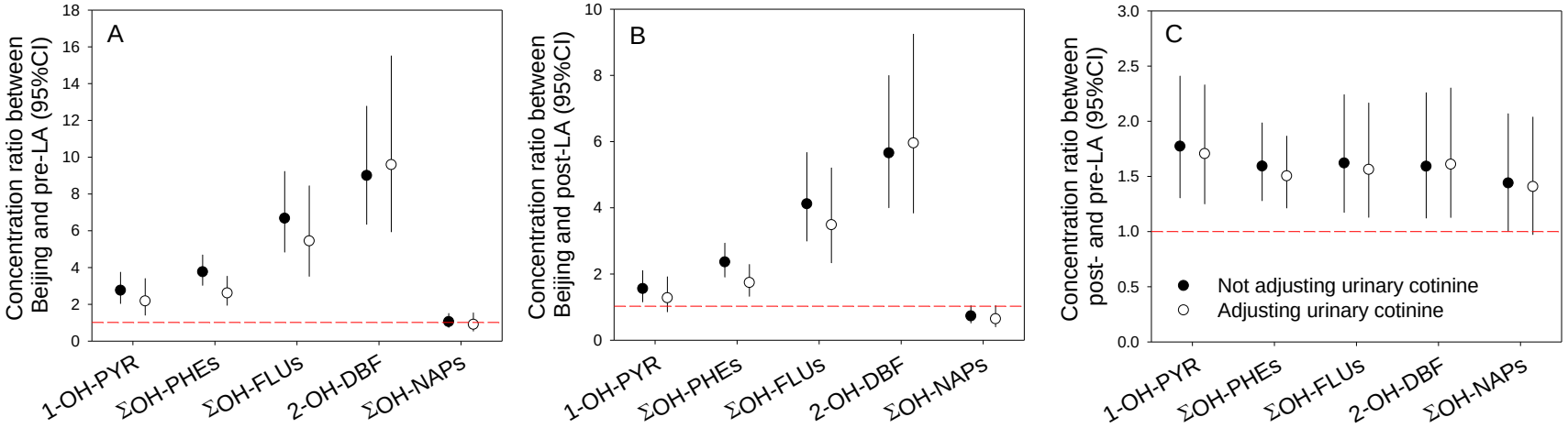

Figure 3.3. Concentration ratios of OH-PAHs between phases. Ratios of concentration of various urinary biomarkers between Beijing and pre-LA (A), Beijing and post-LA (B), and post- and pre-LA (C) with and without adjustment for urinary cotinine. Dashed red line indicates y=1.

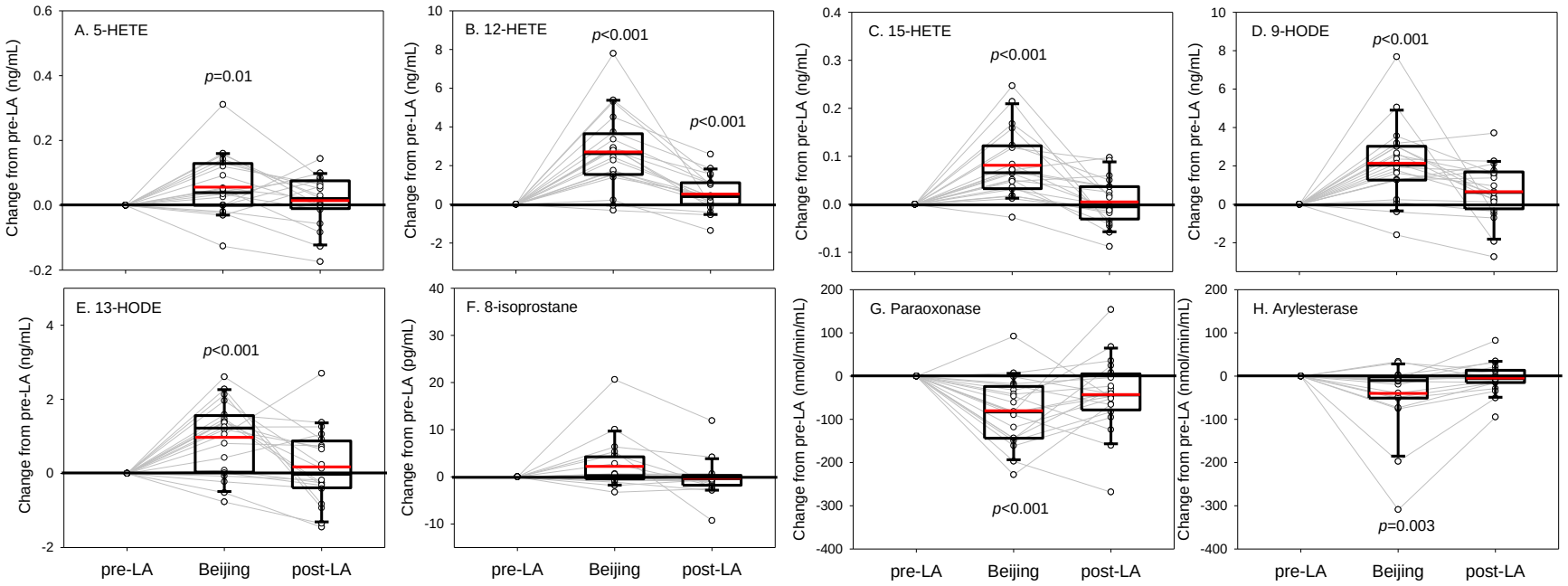

Figure 3.4. Changes in circulating biomarkers of lipid peroxidation (Panels A-F) and PON1 activity (Panels G and F) from pre-LA to Beijing and post-LA. (Open circle represents each subject connected by grey lines. Only subjects who has completed all the three sample collections were included. All the *p*-values indicate significant differences from pre-LA (FDR<0.05 after Benjamini-Hochberg adjustment); the solid horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25th-75th percentiles, and the whiskers represent the 10th and 90th percentiles.)

Table 3.1. Summary of circulating biomarkers and the comparison between pre-LA, Beijing, and post-LA samples

Biomarkers, unit	Geometric mean (interquartile range)			*p*-value for the difference [a]		
	pre-LA (n=23)	Beijing (n=25)	post-LA (n=24)	pre-LA vs. Beijing	post-LA vs. Beijing	pre-LA vs. post-LA
Lipid peroxidation						
5-HETE, ng/mL	0.06 (0.02 - 0.12)	0.11 (0.07 - 0.20)	0.07 (0.05 - 0.11)	0.007*	0.04	0.47
12-HETE, ng/mL	0.29 (0.08 - 0.60)	3.17 (2.11 - 4.79)	0.66 (0.45 - 1.21)	<0.001*	<0.001*	0.002*
15-HETE, ng/mL	0.09 (0.08 - 0.13)	0.17 (0.13 - 0.22)	0.10 (0.09 - 0.12)	<0.001*	<0.001*	0.49
9-HODE, ng/mL	2.70 (2.14 - 3.48)	4.74 (3.95 - 5.27)	3.28 (2.40 - 4.74)	<0.001*	<0.001*	0.04
13-HODE, ng/mL	1.96 (1.55 - 2.63)	2.92 (2.47 - 3.39)	2.10 (1.58 - 2.69)	<0.001*	<0.001*	0.5
8-isoprostane, pg/mL	2.88 (1.37 -5.10)	3.46 (1.67 - 7.38)	2.85 (1.85 - 3.46)	0.05	0.12	0.73
PON1 activity						
Paraoxonase, nmol/min/mL	832 ± 333[a]	783 ± 291	780 ± 295	<0.001*	0.03	0.01
Arylesterase, nmol/min/mL	266 ± 73	227 ± 61	264 ± 61	0.002*	0.005*	0.78
Systemic inflammation						
CRP, μg/mL	0.69 (0.24 - 1.15)	1.37 (0.70 - 4.07)	0.67 (0.30 - 1.24)	0.04	0.02*	0.85
Fibrinogen, μg/mL	0.43 (0.27 - 0.48)	0.64 (0.55 - 0.81)	0.40 (0.26 - 0.47)	0.03*	0.007*	0.68
vWF, μg/mL	5.79 (4.44 - 11.9)	9.12 (6.77 - 13.6)	7.08 (4.73 - 10.6)	0.07	0.31	0.42
Cholesterol level						
Total cholesterol, mg/dL	84.9 (69.6 - 103)	80.5 (65.6 - 94.0)	90.5 (71.8 - 108)	0.59	0.24	0.54
HDL, mg/dL	32.1 (29.0 - 39.2)	31.7 (26.2 - 40.3)	33.0 (28.4 - 39.1)	0.75	0.49	0.71

* FDR<0.05 after Benjamini-Hochberg adjustment

[a.] mean ± standard deviations

[b.] NA: not applicable

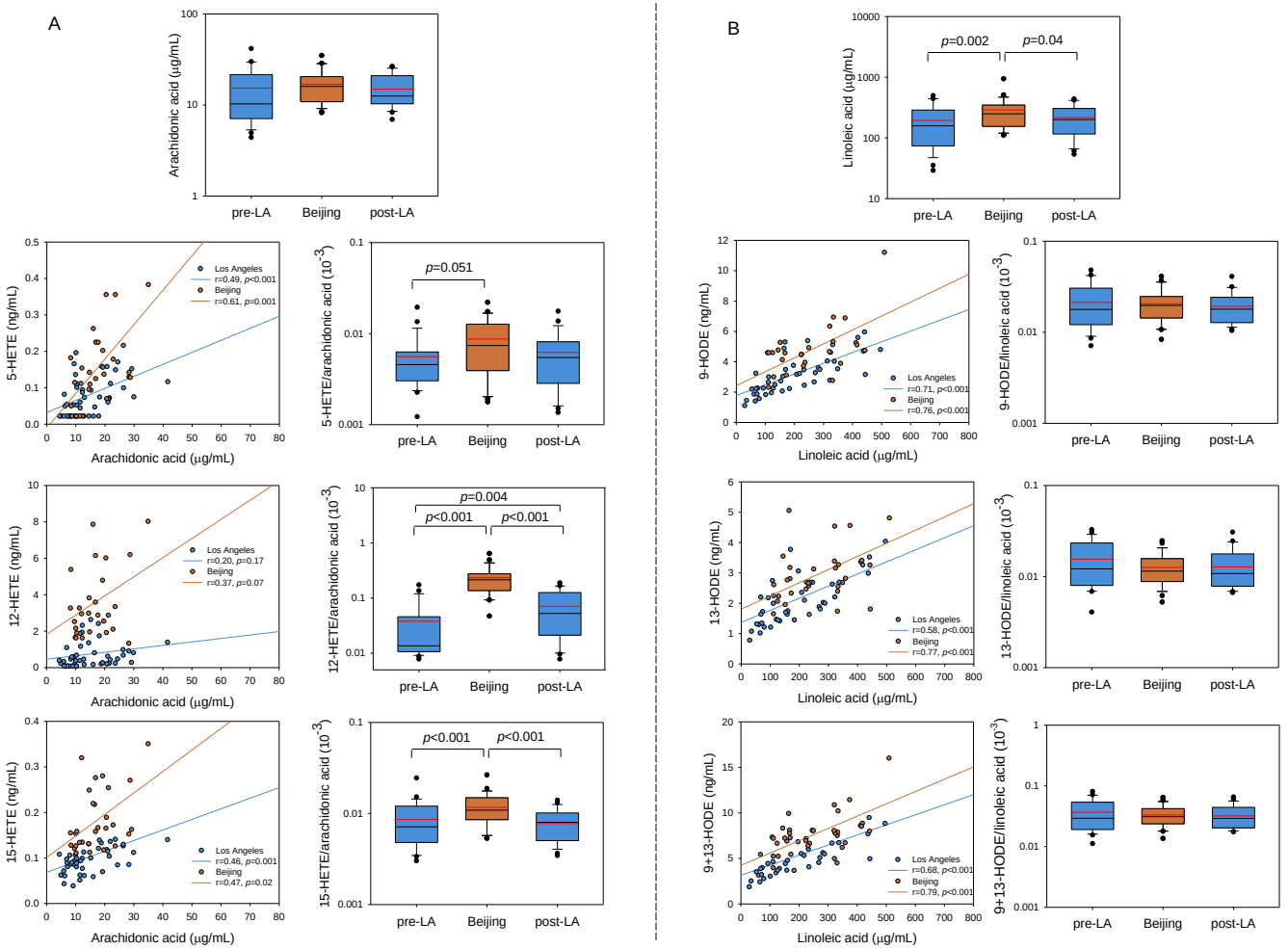

Figure 3.5. Relationship of HETEs (Panel A) and HODEs (Panel B) with arachidonic and linoleic acids, respectively. Correlations were tested by linear regressions. Blue and orange plots indicate data from Los Angeles and Beijing samples, respectively. The black solid horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25th-75th percentiles, and the whiskers represent the 10th and 90th percentiles.

Plasma levels of paraoxonase and arylesterase enzymatic activities were significantly decreased in Beijing as compared with pre-LA (FDR<0.05), suggesting an impairment of PON1, an important antioxidant enzyme associated with HDL.[82] Returning to Los Angeles improved both paraoxonase and arylesterase activities, however, the paraoxonase activity in post-LA was still lower than in pre-LA. The arylesterase activity was negatively correlated with 12- and 15-HETE (p<0.05, Figure 3.6).

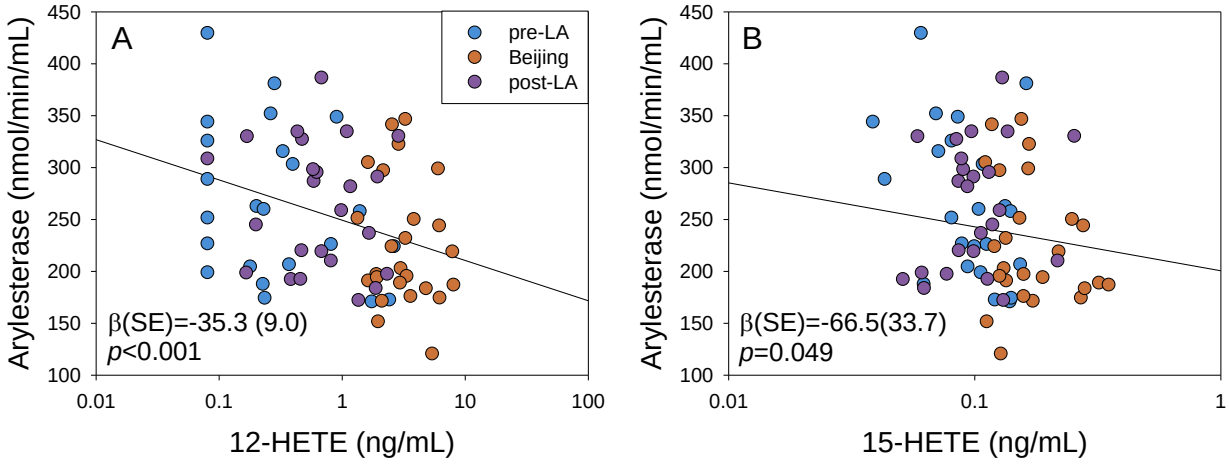

Figure 3.6. Associations of plasma arylesterase activity with 12-HETE (Panel A) and 15-HETE (Panel B) concentrations. The associations were tested by a mixed-effect model with random intercepts of subjects and phases.

We also assessed several CV biomarkers that are currently used in clinical settings (Table 3.1). Significantly higher levels of fibrinogen were observed in Beijing as compared with pre-LA (FDR<0.05). The levels of CRP also increased in Beijing (p<0.05), but the difference became insignificant after multiple comparisons adjustment. These results suggest the induction of systemic proinflammatory and procoagulant effects. No differences in CRP or fibrinogen were observed between pre- and post-LA. A similar trend was observed for vWF, but the differences were not statistically significant. Likewise, levels of total and HDL cholesterol remained unchanged during the entire study period.

Remarkably, all lipid peroxidation biomarkers, except 12-HETE, were significantly associated with at least one of the urinary metabolites of the non-naphthalene PAHs (p<0.05, Figure 3.7). Significant associations were also observed between CRP and urinary ΣOH-FLUs and 2-OH-DBF. In comparison, urinary cotinine only exhibited a positive correlation with 13-HODE, and correlation with 12-HETE that was negative. Thus, the associations of OH-PAHs with CV biomarkers were unlikely due to the collinearity between OH-PAHs and passive smoking.

3.4. Discussion

This is the first human study to reveal alterations in oxidative metabolism of PUFAs and PON1 activity in association with exposure to PAHs, whose likely dominant source is ambient air pollution. We used a natural experiment – arising from the travel of healthy young adults from a less polluted city (Los Angeles) to a city with markedly higher ambient levels of air pollutants (Beijing) – to identify sensitive and novel biomarkers that likely mediate the pro-oxidative and pro-inflammatory effects of subacute (≥ 6 weeks) exposure to environmental pollutants.

The induction of lipid peroxidation in Beijing was supported by increases in a series of oxidative derivatives of arachidonic (i.e. HETEs and 8-isoprostane) and linoleic acids (i.e. HODEs) (Figure 3.4). Thus, increased HETEs and HODEs suggest exacerbation of endogenous lipid peroxidation due to activation of the 5- and 12/15 LOX-mediated pathways. These results are consistent with our previous experimental studies where ApoE$^{-/-}$ and/or LDLR$^{-/-}$ mice, exposed to diesel exhaust or ambient ultrafine particles exhibited increased circulating levels of the same HETEs and HODEs.[17,27] In those studies, we also documented activation of the 5-LOX pathway in the liver by increased mRNA and protein levels of 5-LOX, in parallel with increased hepatic levels of 5-HETE.[17] On the other hand, the increase in 8-isoprotane in Beijing was statistically insignificant , which does not support exacerbation of non-enzymatical oxidation pathways.

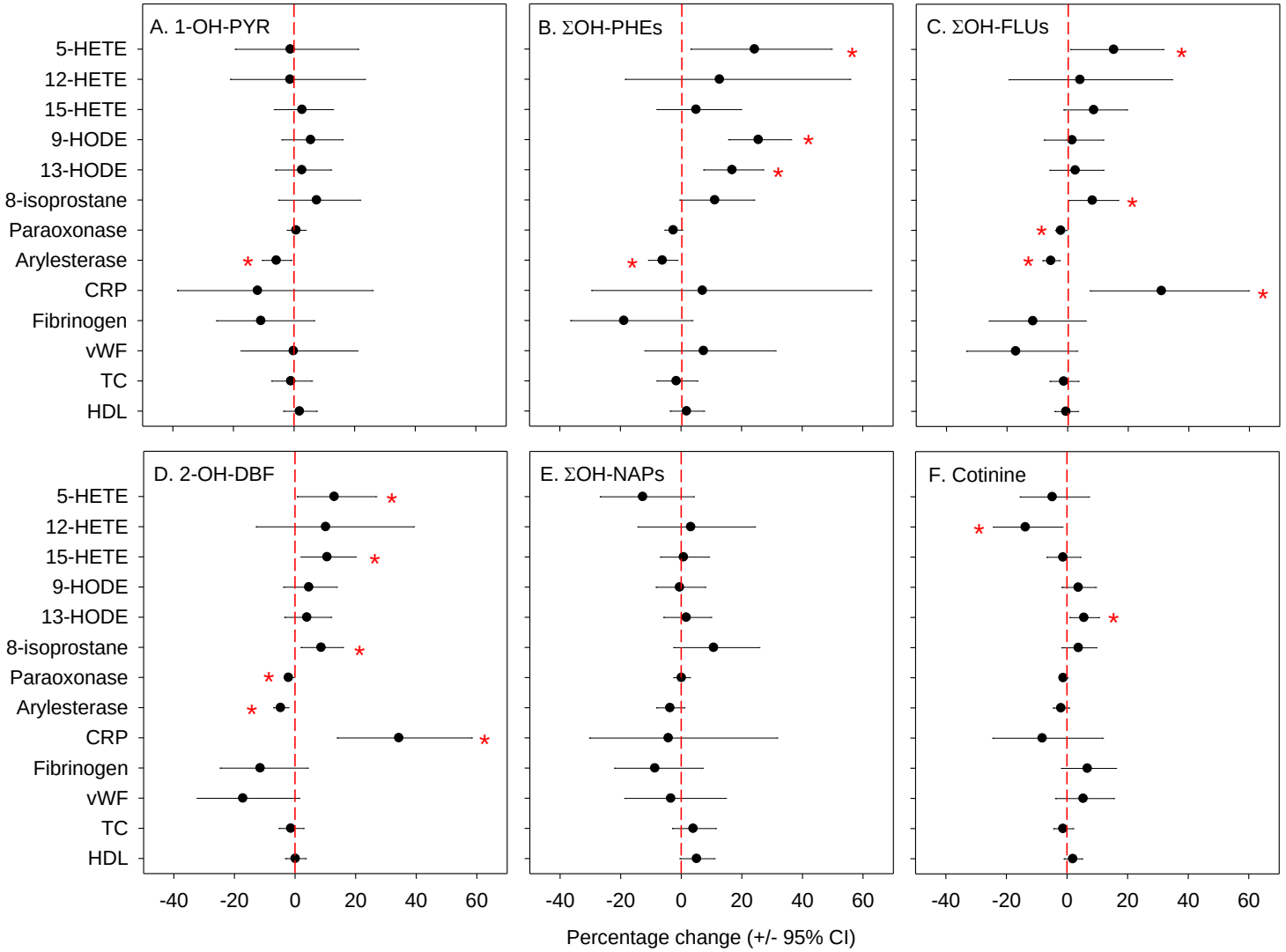

Figure 3.7. Percentage change of cardiovascular biomarkers associated with a one-fold increase in urinary OH-PAHs (Panels A-E) and cotinine (Panel F). (* indicate $p<0.05$ tested by mixed-effect model with random intercepts of phase and subject.)

Traveling to Beijing resulted in decreased PON1 activity (Figure 3.4) that might also contribute to increased lipid peroxidation. The inhibition of PON1 in Beijing might be due to protein post-translational modifications, as the specific activity (i.e. activity per unit protein) of arylesterase, instead of PON1 protein concentration, was significantly lower in Beijing as compared to pre-LA ($p<0.05$, Figure 3.8). On the other hand, both paraoxonase and arylesterase specific activities increased in post-LA as compared with Beijing ($p<0.001$), indicating the recovery of PON1 functionality after returning to Los Angeles. Furthermore, we observed negative associations between oxidative biomarkers (i.e. 12-HETE and 15-HETE) with arylesterase activity, suggesting that PON1 functional changes were likely due to oxidative modifications. This is consistent with previous studies showing that lipid peroxidation inhibits PON1 activity by bonding with available free cysteine reesidues.[83,84]

LOX and PON1 are involved in the early stages of atherosclerosis [24,82,85], and our previous studies have shown that both are affected by environmental exposures in animals.[17,27] While the timeframe in the current study was too brief to assess an impact on atherosclerosis, our results infer the relevance of these pathways among healthy young adults and might shed light on how atherosclerosis is triggered and exacerbated by air pollution in early stages. Moreover, we found that traveling to Beijing resulted in increased levels of CRP and fibrinogen, suggesting the induction of systemic proinflammatory and procoagulant changes as well, which might also associate with higher risk for atherosclerosis development.

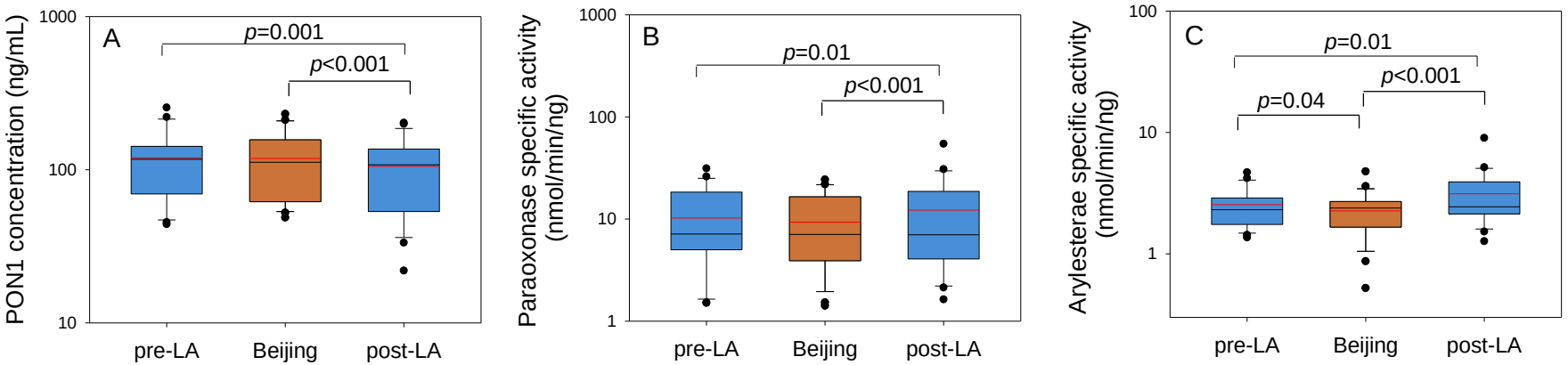

Figure 3.8. Plasma PON1 concentration (Panel A), specific activities of paraoxonase (Panel B) and arylesterase (Panel C) in pre-LA, Beijing and post-LA. Blue and orange boxes indicate data from Los Angeles and Beijing samples, respectively. The solid black horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25^{th}-75^{th} percentiles, and the whiskers represent the 10^{th} and 90^{th} percentiles. $p<0.05$ was considered significant.

Our findings extend previous epidemiological studies that have linked air pollution with increased CV mortality.[42] Although the CV effects in this study may result from exposure to a mixture of PM and gas pollutants, most CV biomarkers were significantly associated with OH-PAHs, suggesting that combustion-originated chemicals might be causative agents. It is important to note that urinary OH-PAHs represented total exposure to PAHs through both inhalation and ingestion.[33] Since dietary PAHs' half-lives in the human body range from 2.5 to 6.1 hours,[45] we carefully controlled ingestion exposure by collecting samples after 8-h fasting in this study. Therefore, it is likely that the increase in urinary OH-PAHs was driven by inhalation exposure to PAHs, present in ambient air and secondhand smoke. Consistent with this, it has been reported that there is a 32.7-fold difference in outdoor airborne non-naphthalene PAHs between Los Angeles and Beijing,[30,31] and we have observed a 8.7-fold difference in urinary cotinine between two cities in this study.

The relevance of HETEs, HODEs, and PON1 activity in CV diseases and their associations with PAHs exposure support their potentials as useful biomarkers for assessing early CV effects induced by air pollution. It appears that HETEs, HODEs and PON1 activity measures have higher sensitivity than 8-isoprostane in response to subacute exposure to environmental pollutants. Importantly, the ICCs of HETEs (<0.17) and HODEs (0.34-0.38) were comparable or even smaller than the ICCs of other CV biomarkers used in clinical settings (Figure 3.9), indicating their small inter-subject variability, and supporting their potential use as novel CV biomarkers. Alternatively, the ICCs of paraoxonase and arylesterase activities were 0.96 and 0.59 respectively, indicating high inter-subject variability, which is consistent with the variability in PON1 activity conferred by genotypic variants.[86]

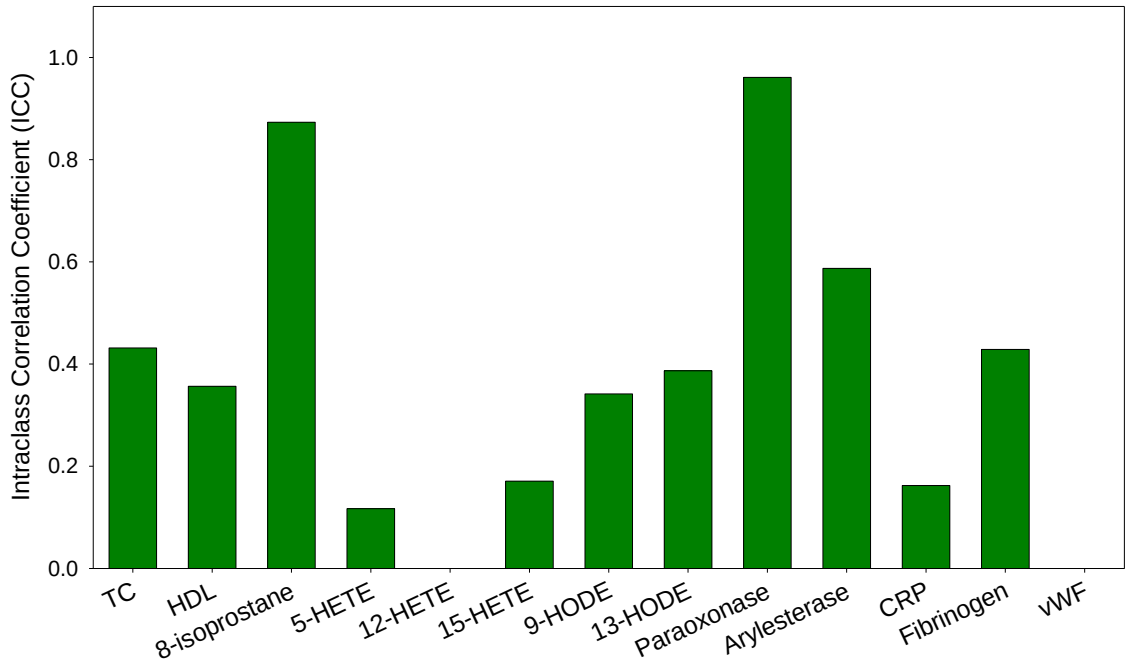

Figure 3.9. Intra-individual correlation coefficients (ICC) of cardiovascular biomarkers

Our results reflect health effects induced by real-world air pollution in Beijing during summertime, but exposures and CV effects could be even greater in other locations and seasons in China. For instance, the levels of ambient $PM_{2.5}$ and urinary OH-PAHs in our study were lower as compared with other cities in China,[87,88] and the air pollution in Beijing is much worse in wintertime due to domestic heating activities.[43] Interestingly, Los Angeles was historically nearly as polluted as Beijing today, but its air quality has drastically improved over the past several decades thanks to the implementation of efficient environmental policies. Although the ambient $PM_{2.5}$ and urinary OH-PAHs in today's Los Angeles were still slightly higher than most cities in high-income countries,[88,89] traveling from Beijing back to Los Angeles led to returns of most CV biomarkers back to baseline, highlighting health benefits that could potentially derive from stricter air pollution control policies. It is also consistent with previous studies that improved air quality

during the Beijing Olympics resulting in decreased circulating levels of CRP and fibrinogen in healthy adults.[90]

Our natural experiment is based on a unique panel who experienced a drastic contrast in exposure to air pollution between two countries. We acknowledge that the study design has limitations because it is difficult to rule out the influence of other factors, such as dietary intake, stress, and exercise that might vary spatially and temporally. Nevertheless, we have found associations between urinary OH-PAHs and CV biomarkers ($p<0.05$), in models controlling the random effects at phase level to adjust temporal and spatial confounders (e.g. city and time), which allowed us to conclude that exposure to PAHs is a likely contributor to the observed CV effects. The study was also limited by a relatively small number of subjects. Despite this, significant CV effects were observed in heathy young adults with potentially more prominent effects among individuals with pre-existing conditions and co-morbidities that increase their susceptibility for CV diseases. In addition, while the effects observed in Beijing here were largely reversed after returning to Los Angeles, we don't know whether repetitive travels could lengthen the persistence of those effects, increasing the risk for developing chronic CV diseases.

In this chapter, we found that healthy young adults traveling from Los Angeles to Beijing developed systemic pro-oxidant and pro-inflammatory changes in association with increased PAHs exposure. The travelers exhibited increased circulating levels of 5-, 12-, 15-HETEs and 9-, 13-HODEs, likely due to activation of 5- and 12/15 LOXs, as well as decreased paraoxonase and arylesterase activities, indicative of decreased in PON1 functionality. These 5- and 12/15-LOX metabolites and PON1 functional measures may serve as sensitive biomarkers of early CV effects induced by air pollution. Our study sheds light on the potential mechanisms linking air pollution to CV diseases.

4. TRENDS IN EXPOSURES TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND BISPHENOL A (BPA) DURING 2012–2017: A COMPARISON BETWEEN LOS ANGELES AND BEIJING

4.1. Abstract

The U.S. and China are among the world's largest emitters and producers of PAHs and bisphenol A (BPA). Health concerns associated with both chemicals have resulted in pollution control policies in both countries, but it has remained unclear to what extent human exposures are thereby affected. To investigate the temporal trends of human exposure to PAHs and BPA in the U.S. and China given different pollution-control policies between the two countries, we recruited 55 apparently healthy young adults who traveled from Los Angeles to Beijing during 2012–2017 and collected a total of 417 morning urine samples before, during, and after the trip. We measured the urinary metabolites of PAHs and BPA with GC/MS. Traveling from Los Angeles to Beijing led to significant increases in urinary metabolites of five PAHs ($p<0.05$) and in urinary BPA ($p<0.001$), which returned to baseline after returning to Los Angeles. The urinary metabolites of PAHs were positively associated with ambient levels of nitrogen dioxide (NO_2) and $PM_{2.5}$ ($p<0.05$) as well as self-reported passive smoking ($p=0.08$) in Beijing but not in Los Angeles. From 2012 to 2017, the levels of PAH metabolites did not change in Los Angeles but significantly decreased in Beijing ($p<0.05$). In contrast, the levels of urinary BPA did not change in Beijing ($p=0.33$) but significantly decreased in Los Angeles by 24% per year ($p<0.05$). These results indicate that people are exposed to higher levels of PAHs and BPA when traveling from Los Angeles to Beijing. Significant exposure reductions were observed for PAHs in Beijing and BPA in Los Angeles, reflecting health benefits from different pollution control policies in the U.S. and China, respectively.

4.2. Introduction

Environmental toxicants present in air, water and food fundamentally threaten the health of the general population.[91] The regulation of these pollutants, however, is multifold and complex due to their various sources and different health effects. Currently, 84,000 chemicals are documented by Toxic Substances Control Act (TSCA), and more than 10,000 are active in the U.S. market.[92] The prioritized chemicals subject to regulation are largely determined by the population's exposure levels relative to the toxic threshold. Previous studies have observed large spatial variations in the population's exposure to chemicals, especially those between high- and low-income countries.[93,94] Therefore, the prioritization of chemical regulations in each country is likely to be different.

As leading causes of the global disease burden, air pollution and passive smoking jointly caused 5.9 million deaths in 2013, 26% of which occurred in China,[1] a country with one of the highest ambient pollutant levels and smoking prevalence in the world.[95,96] Notably, the frequent occurrence of haze episodes in China with impaired visibility has caused widespread public concern.[97] As a milestone of air pollution control in China, the State Council of China issued the Air Pollution Prevention and Control Action Plan (APPCAP) in 2013 to reduce the emissions of air pollutants from various sources, including traffic exhaust, coal burning and industrial processes.[98] Likewise, tobacco controls were also implemented by several cities in China, such as Beijing.[99]

Air pollution and tobacco smoke are both mixtures abundant in PAHs, which have been shown to have significant health effects.[78,100] PAHs are a group of airborne chemicals with more than two fused aromatic rings that are unintentionally emitted by incomplete combustions (e.g., traffic emissions and smoking).[12] The total emission of PAHs in China was 106 Gg in 2007, ranking 1st worldwide and contributing to 21% of global emissions.[32] The emission of PAHs in China was projected to decline in recent years due to fuel quality upgrades. In comparison, current

PAH emissions are much lower in developed countries, such as the U.S. (i.e., 8.5 Gg in 2007), due to the historical pollution-control policies that have been in place for the past 40 years.[32]

In recent years, exposure to BPA has become an important public health concern since studies have shown its adverse health effects at low doses.[101] Unlike PAHs, BPA is intentionally synthesized to produce polycarbonate plastics and epoxy resins.[102] The U.S. has one of the largest BPA manufacturers in the world. In 2007, the production capacity of BPA in the U.S. was 1075 Gg, approximately 6.4-fold of that in mainland China.[102] The results from the National Health and Nutrition Examination Survey (NHANES) in 2003–2004 suggest ubiquitous exposure to BPA among populations in the U.S.,[103] primarily through the usage of food packages and plastic bottles.[104] The concerns of the adverse health effects of BPA, especially on infants and children, have led to nationwide bans of BPA in baby bottles in many countries, including the U.S. and China.[105]

Although extensive actions have been made to reduce the emission of PAHs or the usage of BPA in commercial products, it is unclear to what extent those efforts have been effective in reducing population exposures. There is also a data gap in the extent to which the differences in chemical production and regulation between countries influence human exposures to PAHs and BPA. In this study, we compared the temporal trends of human exposure to PAHs and BPA in the U.S. and China by following 55 college students who traveled between Los Angeles and Beijing from 2012 to 2017. By identifying potential driving forces of the temporal trends in each country, we aim to achieve a better understanding of how chemical regulations may influence human exposures.

4.3. Method

Participants. Participants were recruited from the group of UCLA students who participated in this summer program during 2012–2017. Demographic information was obtained from all participants. The study purpose and the risks were explained to all participants and written informed consent was obtained from all participants. The study was performed in accordance with guidelines and approval of the Institutional Review Boards of both UCLA and PKU.

For each participant, multiple morning urine samples at more than one-week intervals were collected in Los Angeles (pre-LA, before departure), Beijing, and in Los Angeles again (post-LA, after returning), with detailed information shown in Table 4.1. The urine samples were not collected until one week after the arrival at the new city to exclude the exposures from the other city since the half-lives PAHs and BPA in the human body are less than one day.[45,106] Each urine collection was coupled with a questionnaire on the last three days' activity patterns related to PAHs, including cooking behaviors (cooking frequency, cooking fuel, and barbecuing), diet (the consumption of barbecue or baked meat), traffic-related activities (driving hours, public transportation usage, and time spent near heavy traffic areas), and passive smoking, as did in Chapter 2.

Exposure assessment. Exposure to PAHs and BPA was assessed by measuring the levels of their metabolites in the urine with a previously established method as described in Chapter 2.[88] Blank samples were prepared for each of the eight urine samples, and negligible contamination was observed. The average recovery of five surrogate standards (i.e., $^{13}C_6$-2-OH-NAP, $^{13}C_6$-3-OH-FLU, $^{13}C_6$-3-OH-PHE, d_9-1-OH-PYR, and d_{16}-BPA) ranged from 65.5% to 95.0%. Concentrations of metabolites from the same PAHs were summed as an exposure indictor for their parent compound. Daily concentrations of ambient pollutants (i.e., $PM_{2.5}$, NO_2, sulfur dioxide (SO_2),

carbon monoxide (CO), and ozone (O_3)) were obtained from air-pollution monitors within 30 km of UCLA (n=4, 2012-2017) and Peking University (n=18, 2014-2017).

Statistical analysis. All reported biomarkers were detected in more than 80% of the samples, with undetected biomarkers assigned a concentration of 50% of the LOQ. We tabulated the means (+/- standard deviations) or geometric means with the interquartile ranges of exposure biomarkers by phase (i.e., pre-LA, Beijing, and post-LA), as appropriate. The difference in exposure biomarkers between phases was evaluated with linear mixed effects models with participants as the random effects. Linear mixed effects models were also used to test the associations of exposure biomarkers with air pollutants and activity patterns after the data were stratified by city. Temporal trends of biomarkers and other variables were tested by linear or logistic regression models. Significance was established for a two-tailed $p<0.05$. All analyses were performed in R (www.r-project.org).

4.4. Results

We recruited 55 participants (27 males and 28 females), most of whom were young Asian nonsmokers (Table 4.1). The average age and BMI were 24.0 ± 7.2 (mean ± standard deviation) years and 21.3 ± 2.3 kg/m^2, respectively. No temporal trend was observed for age, BMI, race, sex, or smoking status from 2012 to 2017 ($p>0.05$).

Levels and temporal trends of exposure to PAHs. Traveling from Los Angeles to Beijing led to significant increases in urinary levels of all OH-PAHs ($p<0.05$), which decreased after returning to Los Angeles to a level that was comparable with that before travel (Figure 4.1). The differences in urinary OH-PAHs between Beijing and Los Angeles ranged from 1.15-fold (95% confidence interval (CI), 1.01 – 1.31) in ΣOH-NAPs to 6.95-fold (95% CI, 6.14 – 7.87) in 2-OH-DBF. Notably, the differences in urinary levels of all OH-PAHs between the two cities remains robust in different years ($p<0.01$), except for ΣOH-NAPs (Figure 4.1).

Table 4.1. Demographic information of study participants during 2012-2017

	Total	2012	2014	2015	2016	2017	p_{trend} [a]
Number of subjects	55	10	14	13	8	10	
Number of samples (Los Angeles/Beijing)	218/199	56/47	48/64	50/35	27/23	37/30	
Age (yr)	24.0 ± 7.8 [b]	23.3 ± 5.8	23.3 ± 5.6	27.8 ± 13.6	22.6 ± 3.2	22.0 ± 2.7	0.86
BMI (kg/m^2)	21.3 ± 2.3	21.1 ± 1.4	21.7 ± 2.8	21.3 ± 2.1	20.1 ± 2.3	22.1 ± 2.4	0.62
Race (Asian/Others)	40/15	7/3	8/6	12/1	6/2	7/3	0.63
Sex (M/F)	27/28	4/6	9/5	3/10	3/5	8/2	0.34
Smoking (yes/no)	2/53	0/10	0/14	0/13	2/6	0/10	0.29

[a.] Temporal trends were tested by simple linear regressions for age and BMI, and logistic regressions for race, sex and smoking status;
[b.] Mean ± standard deviation

We observed significant decreases in urinary 2-OH-DBF (by 16%/yr, $p<0.05$), ΣOH-FLUs (by 19%/yr, $p<0.05$), and ΣOH-PHEs (by 17%/yr, $p<0.05$) in Beijing from 2012 to 2017 (Table 4.2). In addition, borderline significant decreases were observed for 1-OH-PYR (by 13%/yr, $p=0.05$) and ΣOH-NAPs (by 12%/yr, $p=0.08$) in Beijing as well. In contrast, there was no significant temporal trend of any OH-PAHs in Los Angeles ($p>0.05$).

Determinants of Exposure to PAHs. Exposure to PAHs was associated with different activity patterns in Los Angeles and Beijing. In Beijing, the major determinants of urinary OH-PAHs were activities related to exposure to air pollution, such as time spent near heavy traffic and in public transportation ($p<0.05$). Additionally, marginally significant associations were observed between urinary OH-PAHs and self-reported time in a passive-smoking environment in Beijing ($p=0.08$). In contrast, the most significant determinant of urinary OH-PAHs in Los Angeles was time spent in barbecuing ($p<0.05$), suggesting the impacts of diet on exposure to PAHs.

These findings are further supported by the associations between urinary OH-PAHs and ambient air pollutants. We found that urinary OH-PAHs were significantly associated with NO_2

and $PM_{2.5}$ in Beijing. Similar associations, however, were not found in Los Angeles (Figure 4.2). Notably, the metabolite of PAHs with a relatively higher vapor pressure (i.e., 1-OH-PYR) was significantly associated with $PM_{2.5}$ in Beijing, whereas those metabolites with lower vapor pressures were significantly associated with NO_2.

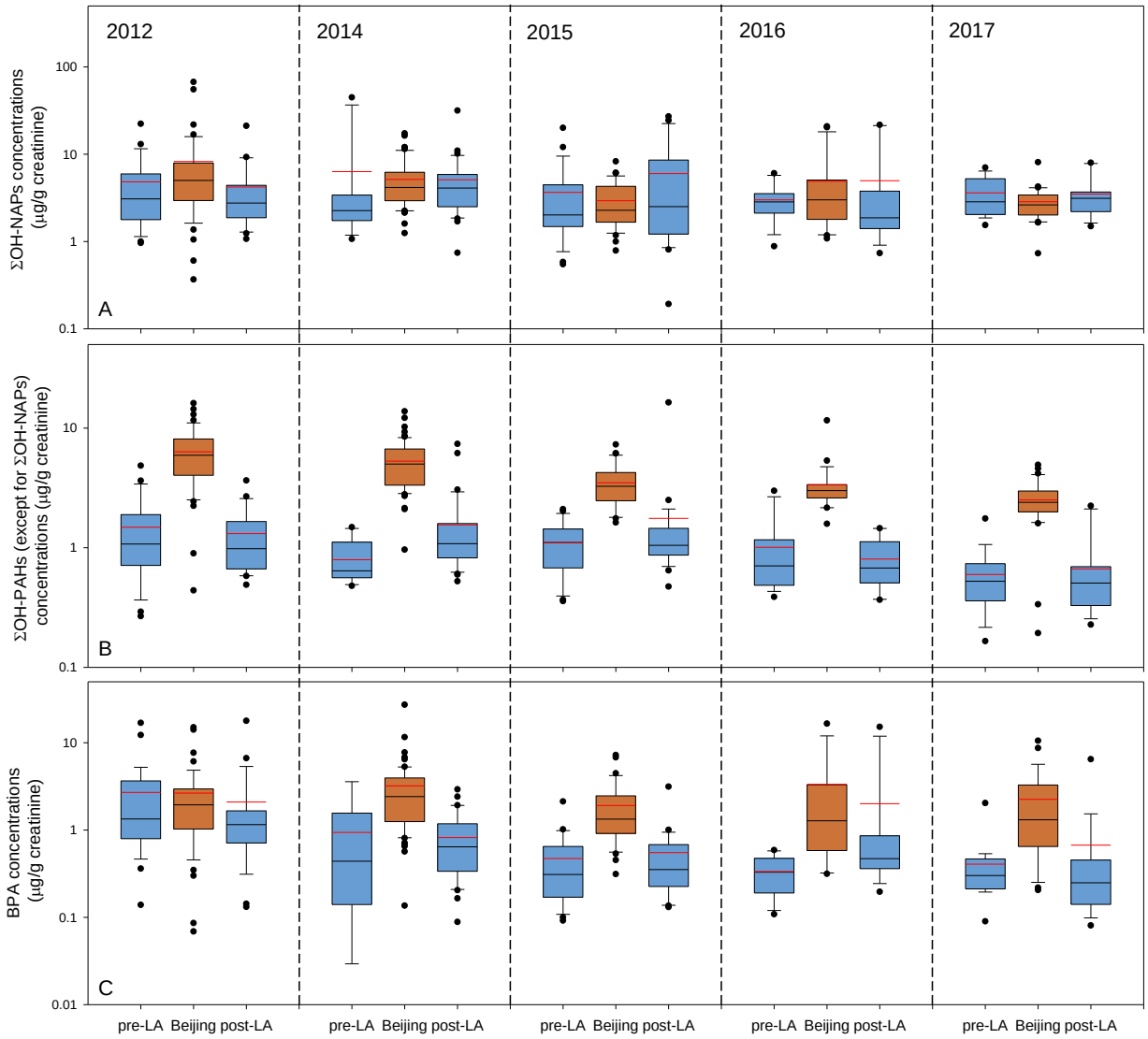

Figure 4.1. Urinary concentration of ΣOH-NAPs (panel A), ΣOH-PAHs (except ΣOH-NAPs, panel B), and BPA (panel C) in pre-LA, Beijing and post-LA during 2012-2017 (Blue and orange boxes indicate data in Los Angeles and Beijing, respectively. The solid horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25^{th}-75^{th} percentiles, and the whiskers represent the 10^{th} and 90^{th} percentiles.)

Table 4.2. Temporal trend of urinary biomarkers between 2012 and 2017 in Beijing and Los Angeles

Biomarkers	2012	2014	2015	2016	2017	Annual Change (%, 95%CI) [b]	*p*-value
Los Angeles							
ΣOH-NAPs	3.31 (1.90-5.57) [a]	3.67 (2.12-5.13)	2.70 (1.36-5.02)	2.67 (1.75-3.35)	3.19 (2.12-4.46)	-3.1 (-14, 9.0)	0.46
2-OH-DBF	0.26 (0.17-0.38)	0.12 (0.07-0.22)	0.15 (0.11-0.20)	0.12 (0.07-0.16)	0.12 (0.08-0.16)	-13 (-28, 4.0)	0.09
ΣOH-FLUs	0.27 (0.16-0.40)	0.24 (0.15-0.32)	0.25 (0.19-0.37)	0.21 (0.15-0.29)	0.14 (0.12-0.20)	-11 (-22, 2.5)	0.08
ΣOH-PHEs	0.40 (0.24-0.54)	0.41 (0.30-0.54)	0.52 (0.38-0.71)	0.29 (0.20-0.41)	0.14 (0.11-0.20)	-16 (-42, 19)	0.21
1-OH-PYR	0.08 (0.05-0.13)	0.11 (0.07-0.17)	0.09 (0.05-0.12)	0.08 (0.05-0.11)	0.04 (0.04-0.07)	-11 (-34, 19)	0.29
BPA	1.36 (0.79-2.26)	0.56 (0.33-1.09)	0.37 (0.20-0.63)	0.45 (0.27-0.52)	0.31 (0.20-0.41)	-24 (-38, -7.3)	0.02
Beijing							
ΣOH-NAPs	5.02 (3.05-7.89)	4.36 (2.94-6.01)	2.50 (1.69-4.21)	3.29 (1.95-4.83)	2.62 (2.03-3.40)	-12 (-26, 3.1)	0.08
2-OH-DBF	1.54 (1.24-2.06)	1.37 (1.02-1.83)	0.74 (0.55-0.99)	0.82 (0.63-0.90)	0.71 (0.65-1.03)	-16 (-28, -1.5)	0.04
ΣOH-FLUs	1.48 (1.20-2.26)	1.43 (0.99-1.94)	0.89 (0.64-1.23)	0.86 (0.48-0.98)	0.48 (0.53-0.84)	-19 (-33, -3.0)	0.03
ΣOH-PHEs	1.76 (1.35-2.64)	1.36 (1.03-1.88)	1.33 (1.02-1.73)	0.95 (0.73-1.15)	0.62 (0.57-0.80)	-17 (-28, -5.4)	0.02
1-OH-PYR	0.27 (0.19-0.45)	0.22 (0.14-0.31)	0.16 (0.11-0.24)	0.20 (0.16-0.28)	0.12 (0.10-0.18)	-13 (-25, 0.5)	0.05
BPA	1.67 (1.07-2.93)	2.27 (1.30-3.93)	1.44 (0.93-2.46)	1.56 (0.61-2.80)	1.31 (0.71-3.02)	-5.8 (-20, 11)	0.33

[a.] Geometric mean (IOR)

[b.] Estimated by simple linear regressions.

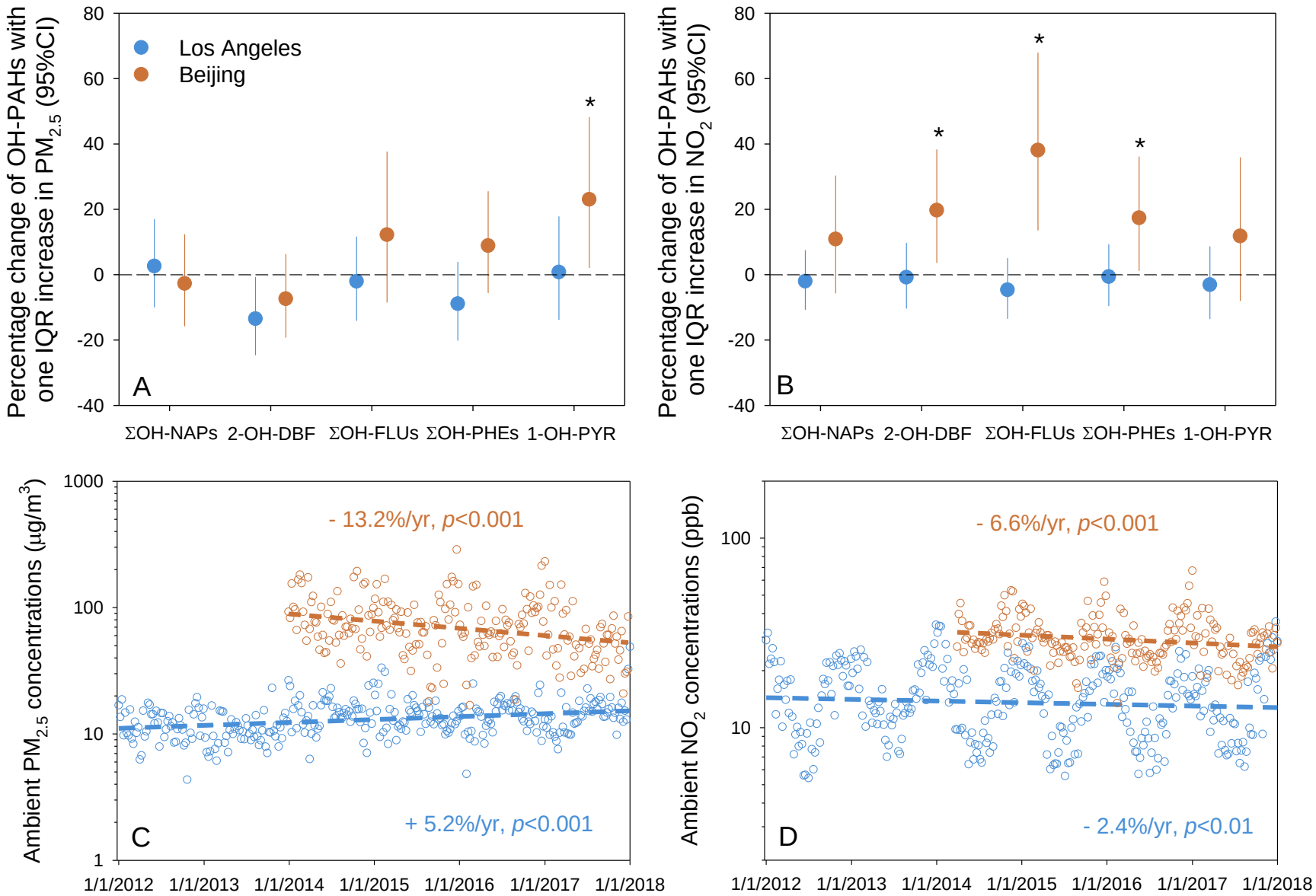

Figure 4.2. Percentage changes of urinary OH-PAHs in associations with one interquartile range changes in past 3-day concentrations of $PM_{2.5}$ (panel A) and NO_2 (panel B) and the temporal trends of $PM_{2.5}$ (panel C) and NO_2 (panel D) during 2012-2017 (* indicates $p<0.05$ tested by mixed-effect models with random effects of subjects; The temporal trends were tested by linear regression models adjusting months; Daily air quality data in Los Angeles and Beijing was obtained from national air quality monitors within 30 km of UCLA (n=4) and Peking University (n=18), respectively)

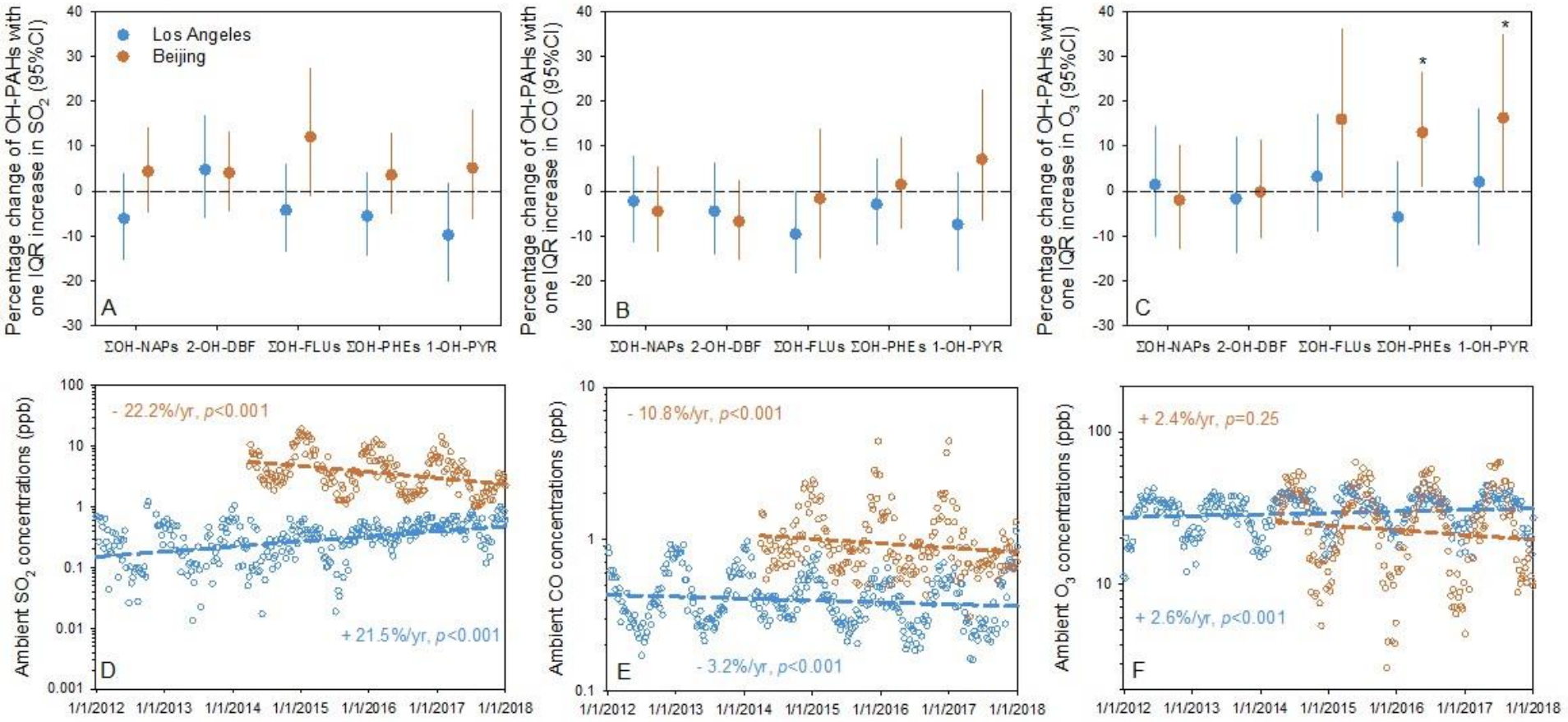

Figure 4.3. Percentage changes of urinary OH-PAHs in associations with one interquartile range changes in past 3-day concentrations of SO_2 (panel A), CO (panel B), and O_3 (panel C), as well as the temporal trends of SO_2 (panel D), CO (panel E), and O_3 (panel F) during 2012-2017

(* indicates $p<0.05$ tested by mixed-effect models with random effects of subjects; The temporal trends were tested by linear regression models adjusting months

Table 4.3. Temporal trend of activity patterns between 2012 and 2017 in Beijing and Los Angeles

Variables	2012	2014	2015	2016	2017	*p*-value [b]
In Los Angeles						
Time in cooking (h)	0.84 ± 0.99[a]	1.43 ± 1.30	1.71 ± 1.93	1.53 ± 1.80	1.49 ± 1.80	**0.02**
Time in barbecuing (h)	0.16 ± 0.52	0.18 ± 0.46	0.12 ± 0.47	0.12 ± 0.59	0.09 ± 0.33	0.25
Barbecue intake (kg)	0.23 ± 0.48	0.18 ± 0.41	0.15 ± 0.43	0.65 ± 2.94	0.14 ± 0.34	0.41
Baked food intake (kg)	0.54 ± 0.78	0.58 ± 0.72	0.44 ± 0.95	0.61 ± 0.89	0.71 ± 0.96	0.99
Driving time (h)	0.69 ± 1.15	1.71 ± 2.38	0.88 ± 1.83	1.20 ± 1.61	0.96 ± 1.91	0.81
Time near heavy traffic (h)	0.63 ± 1.00	0.41 ± 0.65	0.55 ± 0.95	0.91 ± 3.00	0.57 ± 0.79	0.70
Time in public transport (h)	0.58 ± 1.22	0.29 ± 0.92	0.88 ± 1.52	0.84 ± 1.07	0.68 ± 1.51	0.41
Time in passive smoking (h)	0.02 ± 0.09	0.10 ± 0.47	0.00 ± 0.00	0.17 ± 0.47	0.11 ± 0.27	0.15
In Beijing						
Time in cooking (h)	0.00 ± 0.02	0.08 ± 0.41	0.30 ± 0.70	0.17 ± 0.49	0.00 ± 0.00	0.47
Time in barbecuing (h)	0.00 ± 0.00	0.23 ± 0.63	0.01 ± 0.09	0.17 ± 0.58	0.07 ± 0.37	0.73
Barbecue intake (kg)	0.24 ± 0.58	0.18 ± 0.51	0.18 ± 0.86	0.40 ± 0.87	0.09 ± 0.19	0.38
Baked food intake (kg)	0.63 ± 0.56	0.90 ± 1.15	0.74 ± 1.46	1.18 ± 1.48	0.72 ± 1.00	0.88
Driving time (h)	0.15 ± 0.66	0.77 ± 1.92	0.47 ± 1.24	0.09 ± 0.42	0.00 ± 0.00	0.38
Time near heavy traffic (h)	3.86 ± 3.52	2.81 ± 2.07	1.58 ± 1.16	2.47 ± 2.75	2.62 ± 2.85	**<0.001**
Time in public transport (h)	2.87 ± 4.13	3.38 ± 2.09	2.11 ± 1.84	2.35 ± 2.74	2.08 ± 2.09	0.09
Time in passive smoking (h)	1.39 ± 2.34	1.08 ± 1.77	0.99 ± 2.04	0.47 ± 0.76	0.72 ± 1.54	**0.03**

[a.] Mean ± standard deviation

[b.] Annul trends was estimated by linear regressions with the adjustment of sex, age, BMI and smoking status.

Significant temporal trends were observed for some determinants of urinary OH-PAHs as well. As shown in Figure 4.2, the ambient levels of $PM_{2.5}$ and NO_2 in Beijing significantly decreased by 13.2% and 6.6% per year, respectively (p<0.001). Although neither SO_2 nor CO was associated with urinary OH-PAHs, both of their ambient levels declined in Beijing (p<0.001), suggesting significant improvement of air quality (Figure 4.3). No significant temporal trend was observed for ozone in Beijing. In contrast, we observed significant increases in ambient levels of $PM_{2.5}$, SO_2 and O_3 (p<0.001) and slight decreases in NO_2 and CO (p<0.01) in Los Angeles. Likewise, a significant decrease in self-reported passive smoking was observed in Beijing (p<0.05) but not in Los Angeles (Table 4.3).

Levels and Temporal Trends of Exposure to BPA. Similar to urinary OH-PAHs, the levels of urinary BPA were significantly higher in Beijing than Los Angeles (p<0.001), while the levels at pre- and post-LA were comparable (Figure 4.1 and Table 4.2). The differences in urinary BPA between Beijing and Los Angeles increased from 1.23-fold (95% CI, 0.82 – 1.85) in 2012 to 4.05-fold (95% CI, 2.75 – 5.97) in 2017, which is mainly driven by a 24%/yr decrease in urinary BPA in Los Angeles. During the same period, no significant temporal trend in urinary BPA was observed in Beijing (Table 4.2).

4.5. Discussion

This is the first human study that simultaneously characterizes the temporal trends of exposure to PAHs and BPA in different countries. Evidence from travelers between Beijing and Los Angeles has demonstrated significant differences in the levels and temporal trends of the exposures between the two countries. We observed higher exposure to PAHs and BPA in Beijing compared with Los Angeles, suggesting severe environmental pollution problems in China. Notably, significant decreases in PAH exposure were observed in Beijing in association with improved air quality. In

contrast, exposure to BPA significantly decreased in Los Angeles but not in Beijing. These different exposure temporal trends between Los Angeles and Beijing might relate to different pollution-control policies in the U.S. and China.

The reduction of PAH exposure in Beijing was significantly associated with improved air quality in Beijing, which is likely driven by the enactment of the APPCAP. The APPCAP targeted to reduce the annual average $PM_{2.5}$ levels in Beijing to ~60 μg/m^3 in 2017.[98] Indeed, we observed that the ambient $PM_{2.5}$ level was reduced by 13.2%/yr in Beijing and reached an annual average level of 61.5 ± 5.4 μg/m^3 in 2017. While the target of the APPCAP was $PM_{2.5}$ and criteria gas pollutants, it may have the cobenefit of reducing PAH exposure due to the similar environmental sources of $PM_{2.5}$ and PAHs. In fact, previous studies have also documented significant decreases in emissions and outdoor air concentrations of PAHs in China after 2013.[32,107] Our results, for the first time, provide experimental evidence in humans, demonstrating the effectiveness of air pollution control in China in reducing human exposure to airborne toxicants.

It should be noted that the sources of PAHs have large spatiotemporal variability in China.[108] Our previous studies in Beijing have found that the predominant PAH source is traffic emissions in the summer. In the winter or in rural areas near Beijing, however, coal and biomass burning are the major sources of PAHs.[43,109] Because the current study was conducted in the summer in Beijing, the decreases in urinary OH-PAHs were likely driven by the control of traffic emissions, which is supported by the positive associations of urinary OH-PAHs with $PM_{2.5}$ and NO_2 but not SO_2 and CO. While the temporal trends of PAH exposure in rural Beijing or in other seasons remain unclear, we expect similar trends, as strict emission controls of coal and biomass burning have been implemented as well.[110] The annual decline rate of SO_2 (-22.2%/yr, Figure 4.3)

was higher than other air pollutants, suggesting a more aggressive emission reduction in coal burning.

Passive smoking, in addition to air pollution, might also contribute to the spatiotemporal pattern of urinary OH-PAHs, given its borderline significant associations with 2-OH-DBF (p=0.08) and 1-OH-PYR (p=0.09) in Beijing. Notably, the self-reported time in a passive-smoking environment was significantly higher in Beijing compared with Los Angeles, suggesting a significant health risk of passive smoking in Beijing, which is likely due to higher smoking prevalence (30.2% in Beijing vs. 14.3% in Los Angeles).[111–113] In 2015, the Beijing government implemented a smoking ban that prohibits smoking in all public indoor areas and outdoor areas of schools and hospitals.[114] Our results confirmed the effectiveness of this policy since significant decreases in passive smoking were observed in Beijing during 2012–2017 (p<0.05) but not in Los Angeles (p=0.31) (Table 4.3). Nevertheless, self-reported passive smoking remained higher in Beijing compared with Los Angeles in 2017, calling for further efforts on tobacco control.

Owing to the historical control of air pollution and smoking,[112,115] the levels of ambient air pollutants and self-reported passive smoking in Los Angeles were lower compared with Beijing and were not associated with PAH exposure. Instead, other pathways (e.g., diet) might contribute to PAH exposure, as evidenced by significant associations between urinary OH-PAHs and self-reported time barbequing (p<0.05). None of the urinary OH-PAHs had significant temporal trends during 2012–2017 in Los Angeles. Notably, the levels of urinary ΣOH-NAPs in Los Angeles were close to those in Beijing (Figure 4.1). Unlike other PAHs that are only emitted by combustion sources, naphthalene also has non-combustion sources in indoor environments, such as mothballs, fumigants and deodorizers.[116] Additionally, 1-OH-NAP is also a metabolite of carbaryl pesticides.[65] Nevertheless, it is unclear to what extent the levels of urinary OH-NAPs were influenced by these non-combustion sources.

The reduction of PAHs emissions, an important group of unintentionally produced toxicants, is clearly beneficial to human health. In contrast, companies profit from the production of BPA, and there are still controversies over its adverse effects on adults.[101,117] Therefore, the actions to phase out BPA might vary between governments and companies and among states and countries depending on their understanding of BPA safety and the associated cost-benefit trade-off. In our study, we observed significantly lower levels of urinary BPA in Los Angeles ($p<0.001$), which continuously decreased from 2012 to 2017 ($p<0.05$). The spatiotemporal patterns of urinary BPA concentrations in our study were consistent with those previously reported among nonoccupational, healthy populations in the U.S. and China (Figure 4.4, detailed information in text and Table 4.A of the Appendix), suggesting a potential relevance of our findings to nationwide regulations. Remarkably, in contrast to the trends of exposure, the production of BPA in U.S. increased by 37% from 1995 to 2014, [118] suggesting the likely reduction of BPA exposure being through the control of exposure pathways, instead of production.

Substantial evidence suggested that infants are more vulnerable to BPA toxicity,[104] leading to nationwide bans of BPA in baby bottles in both the U.S. and China (Figure 4.4). Comparable policies to reduce the exposure among adults, however, are still lacking due to insufficient evidence on the adverse effects on adults at current levels of BPA.[117] Nevertheless, extensive efforts have been made by manufacturers and local governments in the U.S. to limit the use of BPA in products contributing to human exposure (Figure 4.4). We suspect that customers' awareness of BPA's potential adverse effects might motivate the phase-out of BPA by manufacturers, albeit with controversies, and, therefore, at least partly drive the decreases in BPA exposure among the U.S. population. In comparison, much less effort has been made by manufacturers in China, which might explain the lack of significant changes in BPA exposure in Beijing. On the other hand, public awareness plays an essential role in air quality improvement in

China, as the implementation of the APPCAP in September 2013 was greatly motivated by the increasing public concerns of air pollution after the frequent haze events in January 2013.[119] Thus, our results imply the importance of increasing the public awareness of environmental exposures.

Our study utilized a research opportunity facilitated by international travel to compare the exposures to short-life chemicals (i.e., PAHs and BPA) between the U.S. and China. This natural study provides a real-world exposure scenario within a well-defined time frame, enabling us to explore environmental policies' impacts on human exposures to pollutants. We acknowledge that our study has several limitations. First, urine samples in different years were collected from different participants, which might contribute to the between-year differences. Nevertheless, the demographics of the participants were comparable across different years, and we observed significant but different trends in the exposures between the two cities. Thus, it is unlikely that the temporal trends of exposures are confounded by the differences in participants. Second, most of the participants in our study were college students and might not be representative of the general population in the U.S. or China. Nevertheless, the homogeneity in the study population allows us to reduce the influences of human activity patterns on exposures, and hence the temporal trends were more likely to be driven by changes in the environment. Finally, we did not assess external exposures in our study, that the exposure pathways remain unclear, which needs to be addressed in future studies.

In this chapter, we found significantly higher exposures to PAHs and BPA in Beijing compared with Los Angeles. The population's exposure to PAHs in Beijing significantly decreased from 2012 to 2017, which was likely due to the strict regulation of air pollutant emissions and smoking. In contrast, significant decreases were observed for BPA exposures in Los Angeles, likely due to extensive efforts from governments and manufacturers. Our finding supported significant but different health benefits of pollution control actions in the U.S. and China.

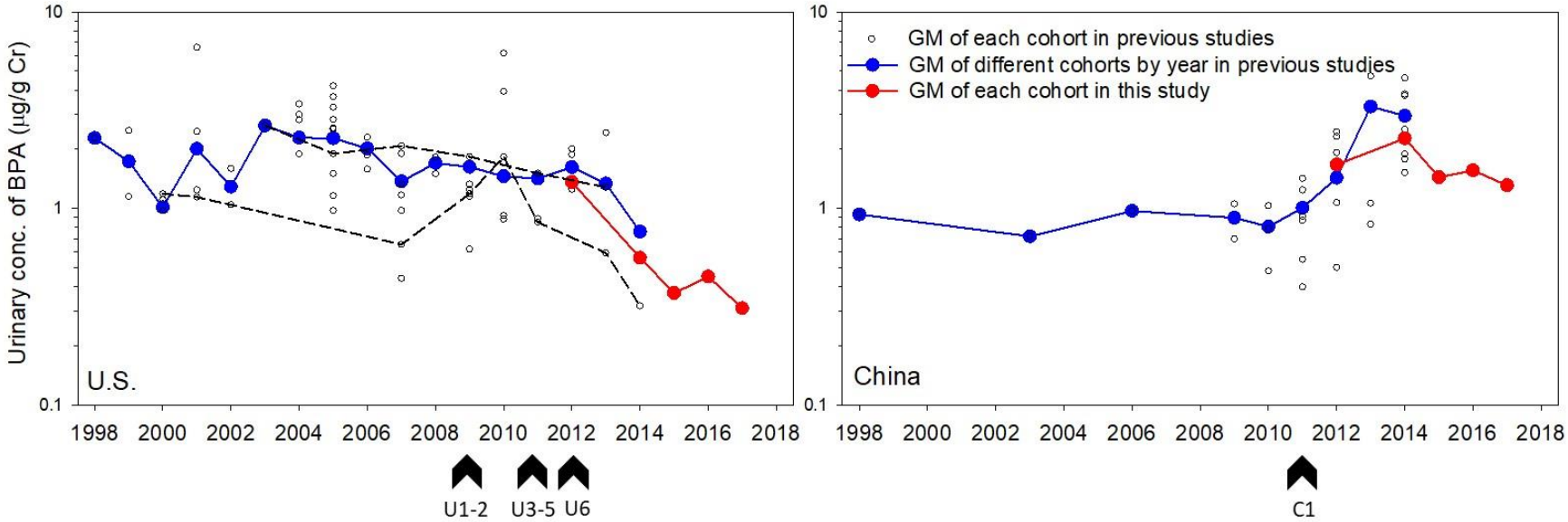

Figure 4.4. Temporal trend of urinary BPA among general population and BPA regulatory policies in the U.S. and China (**U1**: 13 states in U.S. enacted BPA restriction since 2009; **U2**: six large manufacturers stopped selling baby bottles containing BPA in U.S. since 2009; **U3**: U.S.'s largest manufacturer of thermal receipt stop using BPA in 2011; **U4**: major food package companies stopped using BPA in 2011; **U5**: FDA sent clear signs to industries about the transition out of BPA in 2011; **U6**: FDA banned BPA in baby bottles and children's drinking cup in 2012; **C1**: Chinese government banned BPA in baby bottles in 2011. White circle indicates median or mean level if GM is not reported by previous studies; blue circle indicates GM weighed by the number of urine samples; dashed lines connect data from the same studies; Inclusion and exclusion criteria of the studies is introduced in the Appendix and detailed information of each study is summarized in Table 4.A)

4.6. Appendix

Literature review. A literature review was performed to explore the temporal trend of BPA exposure in U.S. and China in the past 20 years (i.e. 1998 – 2018). The publications were identified by searches with chemical formulas in SciFinder, and keywords in Web of Science and Google Scholar databases, as well as reference listed in the identified publications. We included studies that measured the urinary concentration of total BPA in U.S. or China among healthy general population in a clearly defined period. We excluded studies on populations with BPA-related occupational exposures, health conditions or age<3, to avoid metabolic impacts on urinary biomarkers and special exposures that distinguish the studied population with the others (e.g. thermal papers and baby bottles). For studies that resulting in multiple publications, we included only one of them. For the included studied, we summarized their population description, sample size, and statistics of population's urinary BPA, as shown in Table 4.A. We assume a log-normal distribution in urinary BPA among each population. Thus, we obtained their geometric mean (GM) or median levels whenever applicable, otherwise mean level was used. We calculated geometric mean of urinary BPA weighted by the sample size, from different studies by years.

Table 4.A. Summary of the studies cited to investigate the temporal trends of BPA exposure in U.S. and China

Population description	Studied period (range)	Studied period (median)	Number of subjects	Number of samples	Statistics	Urinary BPA levels (μg/g Cr)	Reference
In U.S.							
School girls in New York	1997-1998	1997	192	141	GM[a]	0.16	[120]
Nurses in U.S.	1996-2001	1998	577	577	Median	2.28	[121]
Women in California	1999-2000	1999	491	866	GM	1.15	[122]
Nurses in U.S.	1996-2002	1999	977	977	Median	2.49	[123]
General population in Atlanta	2000	2000	79	79	GM	1.19[b]	[124]
Pregnant women in New York city	1998-2002	2000	173	173	GM	1.11[b]	[125]
Women in Salinas	1999-2000	2000	402	730	GM	0.97[b]	[126]
General population in Atlanta	2001	2001	67	67	GM	1.14[b]	[124]
Children in Ohio	2001	2001	81	81	GM	6.6	[127]
Women in Northwest U.S.	2001-2002	2001	287	287	Median	1.24	[128]
Nurses in U.S.	2000-2002	2001	393	393	Median	2.46	[121]
Men in four U.S. cities	2000-2005	2002	375	375	GM	1.04[b]	[129]
Pregnant women in New York city	1998-2006	2002	375	375	GM	1.59	[130]
General population in U.S.	2003-2004	2003	2517	2517	GM	2.64	[131]
Children in New York city	2004	2004	35	159	GM	3.4	[132]
Pregnant women in Cincinnati	2003-2006	2004	389	1100	GM	1.89	[133]
Children	2003-2006	2004	593	593	Median	2.82	[134]
Girls in U.S.	2004-2005	2004	90	90	GM	3	[135]
General population in U.S.	2005-2006	2005	2548	2548	GM	1.9	[131]
Women in North Carolina	2004-2005	2005	34	67	Median	0.97[b]	[136]
General population in Massachusetts	2004-20066	2005	82	217	GM	1.16[b]	[137]
General population in U.S.	2005	2005	8	328	GM	1.5[b]	[138]
Children in New York city	2001-2010	2005	408	408	GM	3.27[b]	[130]
Children in New York City	2001-2010	2005	401	401	GM	2.83[b]	[130]
Children in New York city	2001-2010	2005	318	318	GM	2.57[b]	[130]
Children in Salinas	2005-200	2005	292	292	GM	3.7	[139]
Girls in three U.S. cities	2004-2007	2005	1101	1101	GM	2.54	[140]
General population in Atlanta	2005	2005	8	427	GM	4.2	[141]
Women in Boston	2004-2008	2006	84	203	GM	1.87[b]	[142]
Women in New York	2005-2008	2006	71	213	Median	1.58	[143]
Women in Buffalo	2005-2007	2006	143	509	GM	2.3	[144]
General population in Atlanta	2007	2007	27	27	GM	0.65[b]	[124]
Pregnant women in Boston	2006-2008	2007	482	1925	Median	1.17[b]	[145]
General population in U.S.	2007-2008	2007	2604	2604	GM	2.08	[131]

Women in Los Angeles	2007-2008	2007	50	50	GM	1.9	[146]
Women in Boston	2004-2009	2007	137	1001	GM	1.33[b]	[147]
Women in Texas and Michigan	2005-2009	2007	501	501	GM	0.98	[148]
Men in Texas and Michigan	2005-2009	2007	501	501	GM	0.44	[148]
Women in Utah and California	2007-2009	2008	374	374	GM	1.5	[149]
Women in Boston	2004-2012	2008	256	673	GM	1.82[b]	[150]
General population in Atlanta	2009	2009	122	122	GM	1.19[b]	[124]
General population in U.S.	2009-2010	2009	2749	2749	GM	1.83	[131]
Women in Boston	2007-2012	2009	239	239	Median	1.15[b]	[151]
Women in Salinas	2009	2009	304	304	GM	1.33	[139]
Pregnant women in New York	2009	2009	10	10	Median	0.62[b]	[152]
Women in U.S.	2009-2010	2009	506	506	GM	1.24[b]	[153]
General population in Atlanta	2010	2010	43	43	GM	1.83[b]	[124]
Firefighters in South California	2010-2011	2010	101	101	GM	1.4	[154]
General population in Illinois	2010	2010	38	38	Mean	2.19[b]	[155]
Pregnant women in San Francisco	2009-2011	2010	112	112	GM	6.16	[156]
Women in Boston	2005-2015	2010	245	417	GM	0.92[b]	[157]
Children in U.S.	2010	2010	153	153	GM	1.71	[158]
Pregnant women in Oklahoma City	2009-2010	2010	72	72	GM	0.88	[159]
General population in San Francisco	2010	2010	20	20	GM	3.94	[160]
General population in Atlanta	2011	2011	95	95	GM	0.85[b]	[124]
General population in U.S.	2011-2012	2011	2489	2489	GM	1.51	[131]
Pregnant women in U.S.	2010-2012	2011	381	381	Median	0.88[b]	[161]
Nurses in U.S.	2011	2011	47	47	Mean	6.75	[162]
University students in Los Angeles	2012	2012	10	56	GM	1.36	this study
Children in Ohio	2012	2012	39	39	Mean	1.82	[163]
General population in Boston	2012-2013	2012	91	91	Median	1.88[b]	[164]
General population in Minnesota	2012	2012	68	68	GM	2.01[b]	[165]
Non-cashiers in North Carolina	2011-2013	2012	21	21	GM	1.25	[166]
General population in Atlanta	2013	2013	141	141	GM	0.59[b]	[124]
General population in U.S.	2013-2014	2013	2686	2686	GM	1.28	[131]
General population in Salt Lake City	2013	2013	50	386	GM	2.42[b]	[167]
University students in Los Angeles	2014	2014	14	48	GM	0.56	this study
General population in Atlanta	2014	2014	42	42	GM	0.32[b]	[124]
Pregnant women in Utah	2014	2014	30	210	Mean	0.85[b]	[168]
University students in Los Angeles	2015	2015	13	50	GM	0.37	this study
University students in Los Angeles	2016	2016	8	27	GM	0.45	this study
University students in Los Angeles	2017	2017	10	34	GM	0.31	this study
In China							
Women in Shanghai	1998-1999	1998	50	50	GM	0.93	[169]

Men in Shanghai	2003-2004	2003	50	50	GM	0.72	[169]
General population in Shanghai	2006-2007	2006	100	200	GM	0.97	[169]
General population in Shanghai	2009	2009	3246	3246	Median	1.05	[170]
General population in Shanghai	2009	2009	1588	1588	Median	0.7[b]	[171]
Pregnant women in Jiangsu	2008-2011	2009	162	162	Median	0.4	[172]
General population in Tianjin	2010	2010	50	50	GM	0.48	[173]
General population	2010	2010	109	109	GM	0.79	[174]
General population in China	2010	2010	116	116	GM	1.03	[175]
School children in Shanghai	2011	2011	259	259	GM	0.4[b]	[176]
School girls in Shanghai	2011	2011	242	242	Median	0.87[b]	[177]
Girls in Shanghai	2011	2011	655	655	Median	1.24	[178]
School boys in Shanghai	2011	2011	671	671	Median	1.42	[179]
Women in Guangzhou	2010-2011	2011	60	60	GM	0.55	[180]
Pregnant women in Nanjing	2010-2012	2011	567	567	GM	0.91	[181]
University students in Beijing	2012	2012	10	47	GM	1.67	this study
Children in east coast of China	2012	2012	718	718	Median	2.45	[182]
School children in eastern China	2012	2012	666	666	GM	2.32	[183]
General population in Hong Kong	2012	2012	140	140	GM	1.92	[184]
Men in Guizhou	2012	2012	560	560	GM	0.5	[185]
Pregnant women in Shandong	2010-2013	2012	496	496	Median	1.07	[186]
General population in South China	2013	2013	94	94	GM	0.83	[187]
General population in Jinan	2013	2013	65	65	GM	1.06	[188]
Pregnant women in Wuhan	2012-2014	2013	339	339	GM	3.9	[189]
Pregnant women in Wuhan	2012-2014	2013	412	412	GM	4.71	[190]
University students in Beijing	2014	2014	14	64	GM	2.27	this study
Rural population in Qingyuan	2014	2014	119	119	GM	3.75	[191]
Rural population in Guangdong	2014	2014	22	22	GM	1.52	[191]
General population in Guangzhou	2014	2014	20	20	GM	1.78	[191]
Children in Guangzhou	2014	2014	96	96	GM	4.61	[192]
University students	2014	2014	6	30	GM	1.89	[193]
Children in Tianjin	2014	2014	256	256	GM	2.52	[194]
Women in Nanjing	2013-2014	2014	300	300	Median	3.81[b]	[195]
University students in Beijing	2015	2015	13	35	GM	1.44	this study
University students in Beijing	2016	2016	8	23	GM	1.56	this study
University students in Beijing	2017	2017	10	30	GM	1.31	this study

[a.] Geometric mean;

[b.] Data reported as ng/mL was converted to μg/g creatinine by using the average creatinine concentrations for the U.S. population of 113.5 mg/dL.

5. CONCLUSIONS

3. Albeit the enactment of APPCAP in China has successfully improved air quality, there is a lack of human evidence indicating the associated health benefit. Our results have shown significant decreases in PAHs exposure in Beijing, in association with air pollutants, while similar decreases were not observed in Los Angeles, suggesting the health benefit of APPCAP in China by reducing PAHs exposures. In addition, we found significant decreases in BPA in Los Angeles, but not in Beijing, suggesting different health benefits of pollution control actions in different countries.

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